Spektroskopie-basierte Authentizitätsprüfung von Speiseölen – Harmonisierungsaspekte

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Der praktische Teil der vorliegenden Arbeit wurde von Dezember 2016 bis Dezember 2019 am Bundesinstitut für Risikobewertung (BfR), Berlin, in der Abteilung „Sicherheit in der Nahrungskette“, Fachgruppe „Produktidentität, Warenketten und Rückverfolgbarkeit“ durchgeführt.

Die vorliegende Dissertation wurde in kumulativer Form angefertigt. Die enthaltenen Forschungsergebnisse wurden vollständig in international anerkannten Fachzeitschriften publiziert, denen die experimentellen Daten, Einzelergebnisse sowie deren Diskussion zu entnehmen sind. Der Fokus dieser Dissertation liegt in der Einordnung der Forschungsergebnisse in den wissenschaftlichen Kontext.

Die folgenden Publikationen und Präsentationen wurden aus den während der Studien für diese Dissertation durchgeführten Arbeiten erstellt.

Publikationen / Präsentationen

Journalbeiträge

Lörchner, C., Fauhl-Hassek, C., Glomb, M. A., Baeten, V., Fernández Pierna, J. A., Esslinger, S. (2024). Comparison of spectroscopic techniques using the adulteration of pumpkin seed oil as example. *Food Analytical Methods*. **(Publikation A)**

Lörchner, C., Horn, M., Berger, F., Fauhl-Hassek, C., Glomb, M. A., Esslinger, S. (2022). Quality control of spectroscopic data in non-targeted analysis – Development of a multivariate control chart. *Food Control*, Volume 133, Part A, 108601. **(Publikation B)**

Lörchner, C., Fauhl-Hassek, C., Glomb, M. A., Baeten, V., Fernández Pierna, J. A., Drescher, S., Esslinger, S. (2023). Towards common useable spectra in non-targeted analysis - a feasibility study by mid-infrared spectroscopy, transfer and correction approaches. *Chemometrics and Intelligent Laboratory Systems*, 1040904. **(Publikation C)**

Vorträge

Lörchner, C. (2019). Qualitätssicherung in der nicht-zielgerichteten Analytik, Abschlussveranstaltung des FoodAuthent-Projektes, Berlin (Deutschland).

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Lörchner, C. (2018). FoodAuthent - Ein Fortschrittsbericht, Next-NMR, Karlsruhe (Deutschland).

Lörchner, C. (2018). Comparable fingerprinting data - an analytical approach towards comprehensive food authentication on the example of edible oils, PreDoc-Symposium, Berlin (Deutschland).

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Lörchner, C. (2017). Vorstellung des Projektes FoodAuthent, Wein- und Fruchtsaft-Analysenkommission, Berlin (Deutschland).

Posterpräsentationen

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Lörchner, C., Fauhl-Hassek, C., Berger, F., Esslinger, S. (2018). Food Fingerprinting – An analytical approach towards comprehensive food authentication (investigation of

hard cheese, edible seed oils and spirits), One Health & Food Safety Congress, Berlin (Deutschland), Posterpreis.

Lörchner, C., Fauhl-Hassek, C., Drescher, S., Esslinger, S. (2018). Untersuchungen der Vergleichbarkeit von Fingerprinting-Daten - Learn to walk before you run, 47. Deutscher Lebensmittelchemikertag, Berlin (Deutschland).

Lörchner, C., Fauhl-Hassek, C., Berger, F., Esslinger, S. (2017). FoodAuthent: Collection, analysis and utilization of analytical food fingerprints, 8. RAFA, Prag (Tschechische Republik).

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Inhaltsverzeichnis

Abkürzungsverzeichnis

AAC	*Administrative Assistance and Cooperation*
ATR	abgeschwächte Totalreflexion
BAM	Bundesanstalt für Materialforschung und -prüfung
BfR	Bundesinstitut für Risikobewertung
BSE	Bovine spongioforme Enzephalopathie
CEN	Europäisches Komitee für Normung
CI	*Confidence Interval*
CRA-W	*Centre Wallon De Recherches Agronomiques*
DA	Diskriminanzanalyse
DIN	Deutsches Institut für Normung
ED	*Euclidian Distance*
ELSD	*Evaporative Light Scattering* Detektor
EN	Europäische Norm
ERETIC	*Electronic REference To access In vivo Concentrations*
EMR	*European Milk Recording*
EU FFN	*European Food Fraud Network*
FID	*Free Induction Decay*
FN	*False Negative*
FP	*False Positive*

FT-MIR	*Fourier Transform-Midinfrared*
FT-Raman	*Fourier Transform*-Raman
GC	Gaschromatografie
GC-FID	Gaschromatografie gekoppelt mit einem Flammen-ionisationsdetektor
GC-MS	Gaschromatografie gekoppelt mit Massenspektrometrie
g.g.A.	geschützte geografische Angabe
HPLC	Hochleistungsflüssigkeitschromatografie
^{1}H-NMR	Protonen-*Nuclear Magnetic Resonance*
IR	Infrarot
ISO	*International Organization for Standardization*
JRC	*Joint Research Center*
KDE	*Kernel Density Estimation*
k-NN	*k-Nearest Neighbours*
KS	Kennard-Stone
LAVES	Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit
LOD	*Limit Of Detection*
LOF	*Local Outlier Factor*
MDL	*Minimum Detection Level*

MIR	*Midinfrared*
MRI	Max Rubner-Institut
MS	Massenspektrometer
NIR	*Nearinfrared*
NRZ	nationales Referenzzentrum
OKG	obere Kontrollgrenze
OPLS-R	*orthogonal projections to latent structures*-Regression
QS	Qualitätssicherung
QS-Probe	Qualitätssicherungs-Probe
OWG	obere Warngrenze
PDS	*Piecewise Direct Standardization*
PLS-DA	*Partial Least Squares-Discriminant Analysis*
PLS-R	*Partial Least Squares*-Regression
PC	*Principal Component*
PCA	*Principal Component Analysis*
PCR	Polymerase-Ketten-Reaktion
$R^2_{Pred.}$	Bestimmtheitskoeffizient *for Prediction*
RMSE	*Root Mean Square Error*
RMSEC	*Root Mean Square Error of Calibration*
RMSECV	*Root Mean Square Error of Cross Validation*

RMSEE	*Root Mean Square Error of Estimation*
RMSEP	*Root Mean Square Error of Prediction*
SNV	*Standard Normal Variate*
SVD	*Singular Value Decomposition*
TN	*True Negative*
TP	*True Positive*
USP	*United States Pharmacopeia*

Kurzzusammenfassung

Die Identifizierung von Verstößen gegen das Lebens- und Futtermittelrecht, einschließlich betrügerischer Praktiken, erfordert wirksame, schnelle und zuverlässige Analyseverfahren. Zudem müssen bereits verwendete Analyseverfahren stetig überprüft, verbessert und neue Verfahren entwickelt werden, um den LebensmittelbetrügerInnen einen Schritt voraus zu sein. Zur Überprüfung der Authentizität eines Lebens- und Futtermittels werden immer häufiger spektroskopie-basierte Analyseverfahren eingesetzt. Hierbei rücken insbesondere nicht-zielgerichtete Ansätze zunehmend in den Fokus der Wissenschaft, welche auf der Erfassung eines chemischen Fingerabdrucks des jeweiligen Lebens- oder Futtermittels in Kombination mit einer chemometrischen Datenanalyse beruhen. Um jedoch standardisierte/harmonisierte nicht-zielgerichtete Analyseverfahren routinemäßig anwenden zu können, müssen noch einige Aspekte und deren aktuelle Limitierungen, insbesondere im Bereich der Validierung und Qualitätssicherung, intensiver untersucht werden. Dazu zählen u. a. eine einheitliche Vorgehensweise zur Validierung des gesamten Verfahrens, einschließlich der multivariaten Datenanalyse, eine laborinterne Qualitätsmaßnahme wie das Führen einer Qualitätskontrollkarte oder die Gewährleistung der Vergleichbarkeit von spektralen Daten. Beim letzten Punkt geht es insbesondere um spektrale Daten, welche mit verschiedenen spektroskopischen Instrumenten nach demselben Messprinzip aufgenommen werden und für die Anwendung einer gemeinsamen Spektraldatenbank in den Laboren (Vergleich der spektralen Daten einer untersuchten Probe mit authentischen Proben) unerlässlich sind.

In der vorliegenden Dissertation wurden daher i) Validierungsstrategien/-parameter am Beispiel der Entwicklung von spektroskopie-basierten Analyseverfahren zur

Identifizierung von Verfälschungen, ii) die Entwicklung einer einheitlichen Strategie zur Qualitätssicherung in der nicht-zielgerichteten Analytik sowie iii) die Gewährleistung der Vergleichbarkeit von spektralen Daten anhand der Beispielmatrix Speiseöle untersucht und Lösungsansätze diskutiert.

Mit dem Ziel spektroskopie-basierte Analyseverfahren zu entwickeln und bestehende Validierungsstrategien/-parameter (u. a. Leistungsfähigkeit und Nachweisgrenzen) zu vergleichen, wurden drei spektroskopische Techniken zum Nachweis von Verfälschungen von Kürbiskernöl durch Zugabe des wesentlich preiswerteren raffinierten Rapsöles untersucht und verglichen. Zu diesem Zweck wurde derselbe Probensatz (reine und verfälschte Proben) mittels *Fourier Transform-Midinfrared* (FT-MIR)-, FT-Raman- und ^{1}H-*Nuclear Magnetic Resonance* (^{1}H-NMR)-Spektroskopie in Kombination mit *Partial Least Squares*-Regression (PLS-R) analysiert. Insgesamt wurden 124 reine Saatenöle und 192 verfälschte Proben (Verfälschungsgrade von 0,5 % w/w bis 90,0 % w/w) mit den drei genannten Techniken untersucht. Die Nachweisgrenzen wurden anhand des *Root Mean Square Error of Prediction* (RMSEP)-Wertes der aus der multivariaten Regression gewonnenen Vorhersagewerte bestimmt. In dieser Studie erzielte die ^{1}H-NMR-Spektroskopie die niedrigste Nachweisgrenze mit 3,4 % (w/w). Für die MIR- und Raman- Spektroskopie wurden Nachweisgrenzen von 4,8 % (w/w) und 9,2 % (w/w) ermittelt.

Bezüglich der Entwicklung von Qualitätssicherungsstrategien bestand das vorrangige Ziel darin, erstmalig eine auf numerischen Daten basierende multivariate Auswertestrategie/Vorgehensweise zur Qualitätskontrolle zu entwickeln, um das gesamte Analyseverfahren einschließlich der Probenvorbereitung sowie der Messung zu überwachen, um zeitliche und/oder instrumentelle Trends oder Ausreißer identifizieren zu können. Auf Basis von numerischen Werten sollten diese Trends und

Ausreißer in einer Qualitätskontrollkarte dokumentiert werden. Um eine multivariate Kontrollkarte für spektroskopische Daten zu erstellen, wurden *Principal Component Analysis* (PCA)-basierte und *outlier score*-basierte Methoden verglichen (am Beispiel von raffiniertem Rapsöl). Die *outlier score*-basierten Methoden *k-Nearest Neighbour* (*k*-NN), *Local Outlier Factor* (LOF), *Kernel Density Estimation* (KDE) und *Euclidian Distance* (ED) zeigten, dass typische Messvariationen in der MIR-Spektroskopie (z. B. Messtemperatur) als Ausreißer identifiziert werden können. Hierfür wurden zuvor berechnete Warn- und Kontrollgrenzen, basierend auf einer definierten Vorperiode von Messungen, herangezogen.

Im dritten Abschnitt der Dissertation wurde im Hinblick auf die Gewährleistung der Vergleichbarkeit von spektralen Daten ein exemplarischer Probensatz von Raps- und Kürbiskernölen mit drei FT-MIR-Spektrometern (MIR 1, 2 und 3) analysiert (identische Probenvorbereitung sowie Messbedingungen). Hierbei wurden Unterschiede in den Absorptionsintensitäten der drei Instrumente festgestellt. Diese Unterschiede beeinflussten die Ergebnisse der PCA und des Zwei-Klassen-*Partial Least Squares-Discriminant Analysis* (PLS-DA) Modells - Kürbiskernöl vs. Rapsöl - und führten zu einer geringeren Sensitivität des Modells und demnach dazu, dass nicht alle Proben der richtigen Klasse zugeordnet wurden. Dies bedeutet, dass Messungen von verschiedenen Instrumenten nicht ohne Anpassungen durch mathematische Korrekturansätze kombiniert werden können, um ein robustes, von mehreren Laboren nutzbares Modell zu entwickeln. Um die spektralen Unterschiede zu minimieren und damit die *performance* Parameter der Vorhersagemodelle zu verbessern, wurden verschiedene mathematische Korrekturansätze untersucht: (i) gerätespezifische Korrekturfaktoren, (ii) Kombinationen verschiedener *pre-processing* Schritte und (iii) *Piecewise Direct Standardisation* (PDS). Die Ergebnisse dieser Studie verdeutlichen,

dass durch Anwendung der Korrekturansätze die Vergleichbarkeit von Spektraldaten verschiedener Instrumente optimiert werden kann, wobei die PDS die vielversprechendsten Ergebnisse zeigt.

Abstract

The identification of food and feed law violations, including fraudulent practices, requires effective, rapid and reliable analytical methods. Furthermore, existing analytical methods need to be constantly reviewed and improved, and new tools need to be developed in order to stay one-step ahead of food fraudsters. Spectroscopy-based analytical approaches are increasingly used to verify the authenticity of food and feed products. In particular, non-targeted approaches are increasingly coming into the focus of science, which are based on the acquisition of a chemical fingerprint of the respective food or feed in combination with chemometric data analysis.

However, to enable their routine application, e.g. in official food control, some aspects and current limitations still need to be investigated intensively, especially in the field of validation and quality assurance. These include, among others, keeping a quality control chart as an internal laboratory quality measure or ensuring the comparability of spectral data recorded with different spectroscopic instruments using the same measurement principle. These aspects are essential for the application of a common spectral database in laboratories (comparison of spectral data of an investigated sample with authentic samples) but have hardly been explored until now.

Therefore, in this thesis i) validation strategies/parameters illustrated by the example of the development of spectroscopy-based analytical methods to identify adulterations, ii) the development of a unified strategy for quality assurance in non-targeted analysis and iii) comparability of spectral data are investigated using the example of seed oils.

With the aim of developing spectroscopy-based analytical methods and comparing existing validation strategies/parameters (including performance and detection limits), three spectroscopic methods for detecting adulteration of pumpkin seed oil by addition

of the much cheaper refined rapeseed oil were investigated and compared. For this purpose, the same sample set (pure and adulterated samples) was quantified using Fourier Transform-Midinfrared (FT-MIR), FT-Raman, and ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectroscopy in combination with partial least squares-regression (PLS-R). A total of 124 pure seed oils and 192 adulterated samples (adulteration levels ranging from 0.5% w/w to 90.0% w/w) were analyzed using the three methods mentioned above. *Minimum Detection Levels* were determined using the root mean square error of prediction (RMSEP) of the predicted values obtained from multivariate regression. In this study, ^{1}H-NMR spectroscopy generated the lowest detection limit of 3.4% (w/w). Detection limits of 4.8% (w/w) and 9.2% (w/w) were obtained for MIR and Raman spectroscopy, respectively.

Regarding the development of quality assurance strategies, the general objective was to monitor the entire analytical procedure, including sample preparation as well as measurement, to enable timely identification of temporal and/or instrumental trends or outliers. Based on numerical values, documentation of these trends and outliers should be documented in a multivariate quality control chart. To create this control chart for spectroscopic data, principal component analysis (PCA)-based and outlier score-based methods were compared. The outlier score-based methods *k*-Nearest Neighbour (*k*-NN), Local Outlier Factor (LOF), Kernel Density Estimation (KDE) and Euclidian Distance (ED) showed that typical measurement variations (e.g. measurement temperature) in FT-MIR spectroscopy can be identified as outliers. For this purpose, previously calculated warning and control limits, based on a defined pre-period of measurements, were used.

In order to ensure comparability, an exemplary sample set of rapeseed and pumpkin seed oil was analyzed using three FT-MIR spectrometers (MIR 1, 2 and 3), an identical

sample preparation and measurement conditions. Differences in the absorbance intensities of the three instruments were found. These differences affected the results of the PCA and the two-class partial least squares discriminant analysis (PLS-DA) model (based on MIR 1 data set) - rapeseed vs. pumpkin seed oil - and resulted in a low sensitivity, which means that not all samples were assigned to the correct class. Therefore, measurements from different instruments cannot be combined without adjustments by mathematical correction approaches to develop a robust model. To minimize the spectral differences and thus improve the performance parameters of the prediction models, several mathematical correction approaches were investigated: (i) instrument-specific correction factors, (ii) combinations of different pre-processing steps, and (iii) Piecewise Direct Standardization (PDS). The results of this study illustrate that by applying the correction approaches, the comparability of spectral data from different instruments can be optimized.

1 Einleitung

Angesichts der zunehmend globalisierten Lebens- und Futtermittelketten ist, neben standardisierten und validierten Analyseverfahren, die vollständige Rückverfolgbarkeit entlang der Lieferkette unerlässlich, um die Qualität, Sicherheit und Authentizität von Lebens- und Futtermitteln zu gewährleisten. Nur so können betrügerische oder irreführende Praktiken/Ereignisse frühzeitig und zuverlässig aufgeklärt werden. Beispiele für solche Praktiken sind Etikettenmanipulationen zur Vortäuschung einer höheren Produktqualität (u. a. Verwendung eines raffinierten Speiseöles anstelle eines nativen Speiseöles) oder die Beimischung minderwertiger Zutaten zu einem hochpreisigen Produkt (u. a. Beimischung von Olivenblättern zu gerebeltem Oregano) (Moore et al., 2012; Spink & Moyer, 2011). Diese i. d. R. wirtschaftlich motivierte Praxis der Verfälschung wird unter dem Begriff Lebensmittelbetrug (*Food Fraud*) zusammengefasst.

Im Zuge der Bovine spongioforme Enzephalopathie (BSE) Krise im Jahr 2000 und der damit einhergehenden Einführung der Verordnung (EG) Nr. 178/2002 wurden einheitliche, EU-weite Standards zur Gewährleistung der Sicherheit von Lebens- und Futtermitteln eingeführt (Verordnung (EG) 178/2002, 2002). Bereits in dieser Verordnung wurde in Artikel 8 (1) folgendes niedergeschrieben: „Das Lebensmittelrecht hat den Schutz der Verbraucherinteressen zum Ziel und muss den Verbrauchern die Möglichkeit bieten, in Bezug auf die Lebensmittel, die sie verzehren, eine sachkundige Wahl zu treffen. Dabei müssen verhindert werden: a) Praktiken des Betrugs oder der Täuschung, b) die Verfälschung von Lebensmitteln und c) alle sonstigen Praktiken, die den Verbraucher irreführen können.“ (Verordnung (EG) 178/2002, 2002).

Um diese Anforderungen des Lebens- und Futtermittelrechts zu überprüfen, wurde u. a. die Verordnung (EG) Nr. 882/2004 verabschiedet, die allgemeine EU-weite Regeln für die Durchführung amtlicher Kontrollen festlegt (Verordnung (EG) 882/2004, 2004). Trotz alledem folgten in den kommenden Jahren unzählige weitere „Lebensmittelskandale“, wie die Melaminkrise bei Säuglingsnahrung 2008 (Pei et al., 2011), der Pferdefleischskandal in Fertigprodukten (z. B. Lasagne) 2013 (Agnoli et al., 2016; Barnett et al., 2016) oder mit dem Pflanzenschutzmittel Fipronil belastete Eier aus den Niederlanden 2017 (van der Merwe et al., 2019). Mit Inkrafttreten der Verordnung (EU) Nr. 2017/625 (Verordnung (EU) 2017/625, 2017) und der Ablösung der bisherigen Verordnung (EG) Nr. 882/2004 (Verordnung (EG) 882/2004, 2004) sollte die Qualität der amtlichen Kontrollen verbessert und vereinheitlicht werden. Zudem wurde die Möglichkeit zur Benennung von Referenzzentren für die Echtheit und Integrität der Lebensmittelkette und für den Tierschutz und die verstärkte risikoorientierte Kontrolle in diesem Bereich geschaffen, die die Bekämpfung von *Food Fraud* in den Fokus der Kontrollbehörden rücken soll (Verordnung (EU) 2017/625, 2017). Auf EU-Ebene wurde bisher noch kein Referenzzentrum benannt. Deutschland hat dagegen als erster EU-Mitgliedsstaat ein nationales Referenzzentrum für authentische Lebensmittel (NRZ Authent) gegründet. Die drei übergeordneten Aufgaben dieses NRZ bestehen darin, ein ExpertInnen-Netzwerk aufzubauen, um 1) Fachwissen zu bündeln und bereitzustellen, 2) (nationale) Datenbanken mit Analyseergebnissen von authentischen Referenzproben aufzubauen und bereitzustellen sowie 3) unterstützend bei der Entwicklung, Validierung, Standardisierung und Auswahl von analytischen Methoden zur Überprüfung der Lebensmittelauthentizität tätig zu sein (MRI, 2021).

Eine rechtlich bindende Definition von *Food Fraud* wird jedoch auch in der neuen EU-Kontroll-Verordnung nicht genannt (Verordnung (EU) 2017/625, 2017). Als Orientierungshilfe, um betrügerische Praktiken von Verstößen gegen geltendes Recht im Lebensmittelsektor zu unterscheiden, hat das *European Food Fraud Network* (EU FFN) vier Schlüsselkriterien festgelegt. Dabei handelt es sich um: i) die Verletzung von EU-Vorschriften, ii) die Täuschung der Verbraucher, iii) den wirtschaftlichen Gewinn und iv) den Vorsatz (European Commission, 2021). In dem Bericht des EU FFN aus dem Jahr 2022 wurden 10 Produktkategorien erfasst, die am häufigsten im System der Amtshilfe und Zusammenarbeit (*Administrative Assistance and Cooperation* - AAC) in Zusammenhang mit *Food Fraud* aufgeführt wurden. Dieses System sammelt Meldungen von EU-Mitgliedstaaten zum Austausch von Informationen über Verstöße und potenziell vorsätzliche Verstöße gegen EU-Rechtsvorschriften, um *Food Fraud* zu bekämpfen. Platz 5 mit einem Anteil von 6,8 % betraf die Produktkategorie "Fette und Öle", wobei die Mehrheit der Meldungen auf Falschetikettierung (eventuelle absichtliche Täuschung) zurückzuführen war (European Commission, 2023). So wurde z. B. raffiniertes Olivenöl als natives Olivenöl deklariert. Um solche Falschetikettierungen identifizieren zu können, bedarf es, zusätzlich zur dokumenten-basierten Rückverfolgbarkeit, spezifischer Authentizitätsprüfungen von Speiseölen. Ein Beispiel für die Erfüllung der vier Schlüsselkriterien ist die Falschdeklaration von Produkten, für die von der EU Qualitätsregelungen festgelegt wurden. Darunter zählt u. a. die geschützte geografische Angabe (g.g.A.) (Verordnung (EU) 1151/2012, 2012). Um eine g.g.A. verwenden zu dürfen, muss mindestens ein Herstellungsschritt (Erzeugung, Verarbeitung oder Zubereitung) in einer spezifischen Region durchgeführt worden sein. In Bezug auf die Produktklasse Speiseöle erregte insbesondere die Untergruppe Olivenöl in den letzten Jahren mediale Aufmerksamkeit, da vermehrt Falschetikettierungen (u. a. nicht korrekte Herkunftsangaben) aufgedeckt

wurden. Es handelte sich dabei um Verstöße, die auf einen *Food Fraud* hindeuten, da 1) ein Verstoß gegen EU-Vorschriften (Verordnung (EU) 1151/2012, 2012), 2) Verbrauchertäuschung (Olivenöl trägt die Angabe einer g.g.A., obwohl kein Herstellungsschritt in der spezifischen Region durchgeführt wurde), 3) eine Gewinnerzielung und 4) ein Vorsatz vorliegen kann. Ein ebenso relevantes, allerdings in den Medien weniger präsentes Beispiel ist das steirische Kürbiskernöl. Bei der Herstellung dieses Speiseöles muss mindestens ein Herstellungsschritt in der Region Steiermark erfolgt sein. Zudem handelt es sich im Vergleich zu anderen Saatenölen (z. B. raffiniertem Rapsöl) beim kaltgepressten Kürbiskernöl um ein hochpreisiges Öl, was auf die erforderliche manuelle Arbeit zur Gewinnung der Früchte, die Extraktion des Öles, die ernährungsphysiologischen Vorteile und die damit einhergehende Bereitschaft von Verbrauchenden das Öl zu einem höheren Preis zu erwerben, zurückzuführen ist (Šamec et al., 2022; Wenzl et al., 2002). Im Gegensatz dazu wird raffiniertes Rapsöl in einem effizienten großindustriellen Verfahren (einschließlich der maschinellen Ernte der Saaten) hergestellt (Wenzl et al., 2002). Durch die hohe Ölausbeute und den geringen Einsatz manueller Arbeit handelt es sich um ein kostengünstiges Produkt. Wird Kürbiskernöl zur Gewinnerzielung mit kostengünstigeren Ölen verfälscht, dann erschweren dem Analytiker die spezifischen organoleptischen Eigenschaften des Kürbiskernöles (starker Eigengeschmack und -geruch sowie eine dunkelgrüne Farbe) die Identifizierung der Zugabe neutraler Speiseöle. Der Geschmack eines raffinierten Rapsöles ist nahezu neutral und beispielsweise die Farbe variiert aufgrund des Raffinationsprozesses von hellgelb bis gelb. Daher lassen sich Geschmack und Farbe beim Mischen mit anderen Speiseölen leicht verbergen. Daher sind Analyseverfahren notwendig, die über die Sensorik hinausgehen, d.h. die anhand der Inhaltsstoffe prüfen, ob es sich um ein reines Kürbiskernöl handelt.

Zur Überprüfung der Authentizität eines Lebensmittels werden zielgerichtete (*targeted*) und nicht-zielgerichtete (*non-targeted*) Verfahren eingesetzt (Esslinger et al., 2014). Im Bereich der Speiseölauthentifizierung werden zurzeit insbesondere klassische, zielgerichtete Analyseverfahren angewendet. Hierzu zählen u. a. die Gaschromatografie (GC) gekoppelt mit einem Flammenionisationsdetektor (FID) zur Bestimmung der freien Fettsäuren bzw. Fettsäuremusters (Castejón et al., 2014; Official Method, 2009/7th; Official Method, reapproved 2017/7th) oder die Hochleistungsflüssigkeitschromatografie (HPLC) in Kombination mit z. B. einem Lichtstreudetektor (*evaporative light scattering* detector) (HPLC-ELSD), insbesondere zur Identifizierung des Profils der Triacylglyceride und zur Quantifizierung der einzelnen Fettsäuren (Salghi et al., 2014). Ein Vorteil dieser zielgerichteten Verfahren ist die Möglichkeit niedrige Nachweisgrenzen (*Limit Of Detection* - LOD) zuverlässig zu erreichen. Jedoch werden hierbei nur spezifische Substanzen bzw. Gruppen von Substanzen identifiziert und ggf. quantifiziert (z. B. Sterole). Dies bedeutet, dass ausschließlich bekannte Substanzen untersucht werden können. Daher sind zielgerichtete Analysetechniken oft für die Aufdeckung von *Food Fraud* ungeeignet, wenn wenig oder keine Kenntnisse zu neuen betrügerischen und täuschenden Praktiken vorhanden sind oder gänzlich unerwartete Zusätze verwendet werden. In diesen Fällen sind Analyseverfahren notwendig, die Abweichungen von der charakteristischen Zusammensetzung oder Verfälschungen mit unbekannten Substanzen identifizieren können.

Um diese Substanzen ermitteln zu können, die nicht charakteristisch für die definierte Lebensmittelmatrix sind, werden insbesondere in den letzten Jahren vermehrt nicht-zielgerichtete Analyseverfahren eingesetzt (siehe auch Kapitel 2.1 - *Nicht-zielgerichtete (non-targeted) Analyseverfahren*). Hierbei wird ein sog. chemischer

Fingerabdruck des jeweiligen Lebensmittels u. a. anhand von spektroskopischen Techniken aufgezeichnet und mittels multivariater Datenanalyse ausgewertet (Antignac et al., 2011). Dieser Fingerabdruck umfasst den gesamten spektralen Bereich einer Lebensmittelprobe und spiegelt demnach das jeweilige Metabolom (je nach spektroskopischer Technik) wider, wobei eine Vielzahl an Proben einer bestimmten Lebensmittelmatrix wie Kürbiskernöl, analysiert werden müssen, um charakteristische, authentische Fingerabdrücke zu erhalten (Abdeckung natürlicher Schwankungen). Nur dann funktioniert der Abgleich einer unbekannten Probe mit einer Datenbank von authentischen Proben.

Um diese Verfahren in der Routine anwenden zu können, gibt es jedoch noch eine Reihe an Herausforderungen, die überwunden werden müssen. Eine dieser Herausforderung liegt in der Etablierung einheitlicher qualitätssichernder Maßnahmen wie das Führen einer Qualitätskontrollkarte, um die allgemeine Zuverlässigkeit der Analyseergebnisse zu gewährleisten. Die Anwendung von nicht-zielgerichteten Analyseverfahren setzt zudem voraus, dass die generierten Fingerabdrücke mit Spektren von authentischen Proben aus einer Datenbank verglichen werden können. Es gibt zwar bereits Datenbanken, die von mehreren Laboren gemeinsam genutzt werden können und eine Vielzahl von Spektren der Lebensmittelmatrices Honig, Wein und Fruchtsäfte sowie Klassifizierungsmodelle beinhalten (Bruker Corporation, 2021; Spraul et al., 2015), jedoch sind diese häufig nicht frei zugänglich und nicht vollständig transparent (unbekannte Datenauswertung), was den Einsatz in den Überwachungseinrichtungen limitiert. Um gemeinsame Datenbanken nutzbar zu machen, müssen diverse Aspekte berücksichtigt werden: Neben authentischen und repräsentativen Proben, die die natürliche Variation innerhalb von Lebens- und Futtermittelmatrices widerspiegeln und eine gültige, anerkannte Referenz für den

Spektrenvergleich darstellen, sind auch umfassende harmonisierte Validierungsstrategien (einschließlich Validierung der statistischen Datenauswertung) erforderlich (Donarski et al., 2019; Esslinger et al., 2014). Eine weitere Herausforderung bei der Anwendung einer gemeinsamen Datenbank ist zudem die Gewährleistung der Vergleichbarkeit der generierten Spektraldaten. Nur, wenn diese Anforderungen erfüllt sind, wird es bei Routinekontrollen von Lebens- und Futtermitteln möglich sein, die Authentizität einer Probe mithilfe von nicht-zielgerichteten Methoden durch Datenbanken und anerkannte multivariate Modellen, die unter Umständen von einem anderen Labor erstellt wurden, genau und korrekt zu bewerten (Dangal & Sanderman, 2020).

Im Rahmen dieser Arbeit wurden die oben beschriebenen Herausforderungen an drei verschiedenen spektroskopie-basierten Techniken mit anschließender Evaluierung der Spektren mittels chemometrischer Verfahren untersucht und diskutiert. Die MIR-, Raman- sowie ^{1}H-NMR-Spektroskopie wurden verwendet, da sie bereits vielfach erfolgreich zur Authentizitätsprüfung von Speiseölen eingesetzt wurden (Alonso-Salces et al., 2022; Berghian-Grosan & Magdas, 2020; Jamwal et al., 2021; Rifna et al., 2022; Xu et al., 2020). Dies erfolgte exemplarisch am Beispiel der Verfälschung von Kürbiskernöl mit raffiniertem Rapsöl. Die Unterscheidung von Kürbiskernöl und Rapsöl wurde gewählt, weil die Ölspektren der botanischen Samen z. B. deutliche Unterschiede im Bereich von 1050 cm^{-1} - 880 cm^{-1} (Biegeschwingungen von konjugierten CH trans-, trans- und cis, trans-olefinischen Gruppen) aufweisen (Beyzi et al., 2019; Guillén & Cabo, 2000, 2002; Rezig et al., 2012); angesichts der großen Unterschiede im Fettsäurespektrum wurde daher angenommen, dass eine 100 %ige Zuordnung zwischen den beiden Sorten möglich ist.

2 Theoretischer Teil

2.1 Definition von Authentizität und Authentizitätsprüfung

Das Wort *Authentizität* bedeutet Echtheit oder auch Glaubwürdigkeit und entlehnt sich aus dem spätlateinischen Wort *authenticus* bzw. dem griechischen Wort *authentikos*. Im Lebensmittelbereich gilt ein Produkt als authentisch, wenn alle Voraussetzungen bezüglich der Deklaration auf dem Etikett erfüllt werden, d. h. die angegebene Zusammensetzung, Art, Herstellungsmethode, botanische oder geografische Herkunft mit dem Inhalt übereinstimmen (Esslinger et al., 2014). Um *Food Fraud* (z. B. die Beimischung von minderwertigen Ölen zu hochpreisigem Speiseöl) aufzudecken und somit die Echtheit eines Lebensmittels zu überprüfen, werden zum einen dokumenten-basierte Kontrollen und zum anderen verschiedene analytische Untersuchungen durchgeführt. Diese werden, wie eingangs erwähnt, in zielgerichtete (*targeted*) und nicht-zielgerichtete (*non-targeted*) Analyseverfahren untergliedert.

Zielgerichtete (targeted) Analyseverfahren

Mit Hilfe der zielgerichteten Analytik können eine oder mehrere vordefinierte Verbindungen/Substanzen detektiert bzw. quantifiziert werden. Dies bedeutet, dass vor der jeweiligen Analyse bereits festgelegt wird, welche Verbindung/Substanz gesucht wird, die spezifisch oder eben nicht spezifisch für die jeweilige Lebensmittelmatrix ist (Ballin & Laursen, 2019). Werden solche Marker identifiziert, werden diese anschließend mit festgelegten Grenzwerten/Höchstwerten oder auch spezifischen Schwankungsbreiten (für die jeweilige Lebensmittelgruppe) verglichen, um zu überprüfen, ob der Gehalt dieses Markers den entsprechenden lebensmittel-rechtlichen Bestimmungen entspricht (Esslinger et al., 2014). Ein Beispiel ist die

Bestimmung der Fettsäureverteilung, die je nach Speiseölsorte variieren kann. Mit Hilfe der GC-FID kann das Fettsäurespektrum (Fettsäurezusammensetzung) ermittelt werden, indem die freien Fettsäuren in Form ihrer Methylester-Derivate getrennt und quantitativ bestimmt werden (Official Method, 2009/7th; Official Method, reapproved 2017/7th). Die Zusammensetzung kann anschließend mit den Schwankungsbreiten verglichen (Leitsätze für Speisefette und Speiseöle, 2020) und lebensmittelrechtlich beurteilt werden.

Neben der Analyse von natürlichen Bestandteilen eines Lebens- oder Futtermittels können auch Verbindungen/Substanzen ermittelt werden, die beim Herstellungs-/Verarbeitungsprozess entstehen können. Hierzu zählt z. B. der Gehalt an Stigmastadien in Speiseölen. Diese Verbindung entsteht durch den Abbau des Pflanzensterins *β*-Sitosterin bei der Raffination (u. a. durch Bleichung) von pflanzlichen Ölen (Gordon & Firman, 2001) und kann demnach zum Nachweis von raffinierten in nativen Speiseölen verwendet werden. Für „natives Olivenöl extra“ und „natives Olivenöl“ wurde ein Grenzwert an Stigmastadien von 0,05 mg/kg festgelegt (Delegierte Verordnung (EU) 2022/2104, 2022). Für andere kaltgepresste Speiseöle existieren diese Grenzwerte nicht, jedoch wird u. a. vom Niedersächsischen Landesamt für Verbraucherschutz und Lebensmittelsicherheit (LAVES) beschrieben, dass bei Gehalten über 1 mg/kg angenommen werden kann, dass eine Wärmebehandlung erfolgt ist (LAVES, 2020). Nach den deutschen Leitsätzen für Speisefette und Speiseöle (Leitsätze für Speisefette und Speiseöle, 2020) ist eine Wärmebehandlung jedoch unzulässig, wenn auf dem Etikett keinerlei Hinweise darauf deklariert sind.

Zudem können mit zielgerichteten Analyseverfahren zum einen exogene Verbindungen nachgewiesen werden, d. h. bekannte Substanzen, die in der spezifischen Matrix von Natur aus nicht vorkommen und zum anderen Substanzen,

die grundsätzlich nicht in einem Lebens- oder Futtermittel enthalten sein dürfen wie u. a. Benzoesäure in Ölen und Fetten tierischen und pflanzlichen Ursprungs (Verordnung (EU) 1129/2011, 2013/001.001; Verordnung (EG) 1333/2008, 2008).

Nicht-zielgerichtete (non-targeted) Analyseverfahren

Bei nicht-zielgerichteten Analyseverfahren wird ein sog. Fingerabdruck einer spezifischen Lebensmittelmatrix in Kombination mit einer chemometrischen Auswertung der gewonnenen, z. B. spektroskopischen Daten für eine umfassende Probencharakterisierung verwendet (McGrath et al., 2018). Die Probenvorbereitung für die nicht-zielgerichtete Analytik sollte so unselektiv wie möglich sein, um Bestandteile der Probe und somit Informationen nicht vor der Messung zu entfernen (Cavanna et al., 2018; Esslinger et al., 2014). Im Gegensatz zu zielgerichteten Techniken sind umfangreiche Probenvorbereitungsverfahren wie Veresterung, Umesterung oder Fettextraktion nicht erforderlich (Castejón et al., 2014). Neben dem Vorteil der Zeitersparnis, werden in der Regel zudem weniger gesundheitsgefährdende Chemikalien verwendet. Des Weiteren können nicht-zielgerichtete Ansätze grundsätzlich auch eingesetzt werden, um unbekannte Substanzen zu detektieren. Wichtiger Bestandteil bei der Auswertung solcher Datensätze und der damit einhergehenden Authentifizierung ist neben der multivariaten Datenauswertung der Vergleich mit Referenzdaten von authentischen Proben, welche in einer Referenzdatenbank hinterlegt sind. Hierbei können Schwankungen/Abweichungen von der Referenz, also authentischen und unverfälschten Proben, ermittelt werden. Hierbei ist wichtig zu erwähnen, dass die natürliche Variation einer Matrix in der Referenzdatenbank so gut wie möglich abgebildet werden muss und je nach Fragestellung Faktoren berücksichtigt werden

müssen, die natürliche Schwankungen innerhalb einer Matrix verursachen können wie z. B. das Erntejahr, die geografische Herkunft, Verarbeitungsprozesse oder auch anthropogene Einflüsse (Donarski et al., 2019). Die Daten können auch univariat ausgewertet werden, indem ein einzelnes Signal herangezogen und die Substanz anschließend quantifiziert und der ermittelte Gehalt mit Werten aus der Referenzdatenbank verglichen wird.

2.2 Spektroskopie-basierte Ansätze in Kombination mit multivariater Datenanalyse

Eine Übersicht der in der vorliegenden Arbeit durchgeführten Vorgehensweise ist in Abbildung 1 dargestellt.

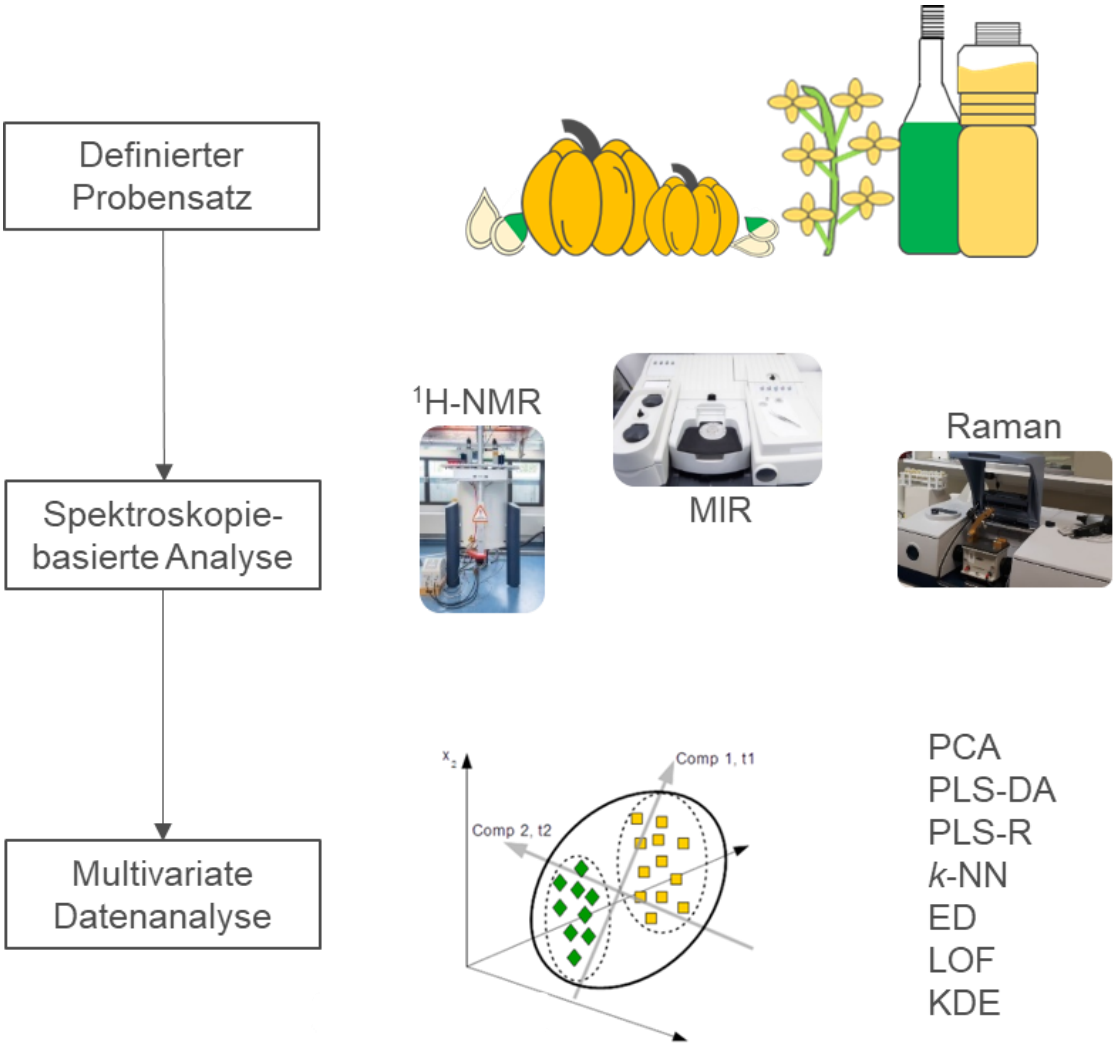

Abbildung 1: Überblick über die eingesetzten spektroskopischen Techniken und die verschiedenen multivariaten Modelle; ^{1}H-NMR: Protonen-*Nuclear Magnetic Resonance*-Spektroskopie, MIR: *Midinfrared*-Spektroskopie, PLS-DA: *Partial Least Squares-Discriminant Analysis*, PLS-R: *Partial Least Squares-Regression*, *k*-NN: *k-Nearest Neighbours*, ED: *Euclidian Distance*, LOF: *Local Outlier Factor*, KDE: *Kernel Density Estimation*.

2.2.1 FT-MIR-Spektroskopie

Die Infrarot-Spektroskopie, welche auf der Anregung höherer Energiezustände der Moleküle beruht, wurde ursprünglich zur Strukturaufklärung und der quantitativen Bestimmung von bekannten Verbindungen (z. B. von Fettsäuren) eingesetzt (Skoog et al., 2017; Vermeulen et al., 2010). Infrarotstrahlung wird der elektromagnetischen Strahlung zugeordnet und erstreckt sich über einen Wellenlängenbereich von 400 cm^{-1} bis 4000 cm^{-1}, wobei die Dimension des Infrarotspektrums üblicherweise in Wellenzahlen ($\tilde{\nu}$ - Wellenzahlbereich von 2500 nm bis 15.400 nm) angegeben wird, da die Wellenzahl direkt proportional zur Energie der absorbierten Strahlung ist (Günzler & Gremlich, 2003). Während der Analyse wird die zu untersuchende Probe elektromagnetischer Strahlung ausgesetzt. Hierdurch kommt es zur Anregung von Molekülschwingungen in Abhängigkeit der schwingenden Massen und der Bindungsstärke. Die auftretenden IR-aktiven Absorptionsbanden geben Aufschluss über funktionelle Gruppen (wie die Streckschwingung von Carbonylgruppen C=O oder von Hydroxylgruppen O-H) in einem Molekül (Bienz et al., 2016) und sind für jede Probe individuell und einzigartig, je nach chemischer Zusammensetzung des jeweiligen Speiseöles (Guillén & Cabo, 1998, 1999; Vermeulen et al., 2010).

2.2.2 FT-Raman-Spektroskopie

Die Raman-Spektroskopie liefert ebenfalls Informationen über Schwingungs- und Rotationszustände von Molekülen, ist jedoch unabhängig von der Wellenlänge der Erregerstrahlung. Unterschiede zwischen der Raman- und IR-Spektroskopie liegen zudem in den physikalischen Grundlagen und in der Anregung der zu untersuchenden Probe (Vandenabeele, 2013). In der Raman-Spektroskopie wird ein intensiver, monochromatischer Laserstrahl zur Anregung von Molekülen angewendet. Wird auf

eine Probe eine Laserstrahlung gerichtet, wird ein wesentlich größerer Anteil des einfallenden Lichts elastisch gestreut (Rayleigh-Streuung) (Bienz et al., 2016; Vandenabeele, 2013). Ein sehr geringer Teil des Laserlichtes wird dagegen unelastisch gestreut (sog. Raman-Streuung). Der Grund für diese Raman-Streuung liegt in der Deformierbarkeit der Elektronenhülle (Polarisierbarkeit) des Moleküls während des Schwingungsvorgangs, wobei das gestreute Licht eine niedrigere oder höhere Frequenz als das einfallende Licht haben kann. Diese Energieübertragung resultiert in der Verschiebung der Wellenlänge des gestreuten Lichts, was als Raman-Verschiebung bezeichnet wird (Vandenabeele, 2013). In einem typischen Raman-Spektrum wird daher diese Raman-Verschiebung gegen die Wellenzahl aufgetragen. Konjugierte Doppelbindungen oder aromatische Gruppen sind stark polarisierbar, da sie ein niedriges Dipolmoment (delokalisiertes Elektronensystem) besitzen und somit IR-inaktiv sind, jedoch Raman-aktiv. Dagegen zeigen chemische Bindungen mit hohem Dipolmoment in der Regel eine schlechte Polarisierbarkeit und sind daher IR-aktiv, aber kaum Raman-aktiv. Die IR- und Raman-Spektroskopie können somit als komplementär betrachtet werden (Vandenabeele, 2013). In verschiedenen Publikationen wurde bereits intensiv die Authentizitätsprüfung von Speiseölen mittels Raman-Spektroskopie beschrieben (Baeten, 2010; Baeten & Aparicio, 2000; Baeten et al., 1998).

2.2.3 ^{1}H-NMR-Spektroskopie

Die NMR-Spektroskopie wird insbesondere in der analytischen Chemie zur Qualitätskontrolle, zur Bewertung der Reinheit und zur Aufklärung unbekannter Strukturen sowie zur Quantifizierung organischer Verbindungen eingesetzt (Günther, 2013). Viele Atomkerne besitzen einen natürlichen Eigendrehimpuls (auch Kernspin)

und rotieren anhand dessen um ihre innere Achse. Durch Anlegen eines externen, starken und statischen Magnetfelds gekoppelt mit einem hochfrequenten magnetischen Wechselfeld wird ein Energietransfer von der Basisenergie auf ein höheres Energieniveau erreicht. Kehrt der Kernspin auf sein Ausgangsniveau zurück, wird die Energie freigesetzt und kann gemessen werden, wobei ein *Free Induction Decay* (FID) erzeugt wird. Diese Funktion der Zeit wird durch Fourier Transformation mathematisch in eine Funktion der Frequenz umgerechnet, was mit einem typischen NMR-Spektrum (Signalintensität gegen die Frequenz) resultiert. Das intramolekulare Magnetfeld eines Atoms beeinflusst die Resonanzfrequenz, und es lassen sich Details der elektronischen Struktur eines Moleküls und seiner funktionellen Gruppen ableiten (Günther, 2013). In der vorliegenden Arbeit wurde die ^{1}H-NMR-Spektroskopie genutzt, welche bereits seit mehreren Jahren in der Speiseölauthentifizierung u. a. zur Bestimmung der Fettsäurezusammensetzung eingesetzt wird (Barison et al., 2010; Castejón et al., 2014; Di Pietro et al., 2020; Guillén & Ruiz, 2003a, 2003b).

2.2.4 Multivariate Datenanalyse

Um die Qualität und damit einhergehend die Authentizität eines Lebensmittels zu beurteilen, wurden früher häufig univariate Ansätze eingesetzt, die sich lediglich auf eine Komponente bzw. Eigenschaft des Lebensmittels (z. B. Textur, Farbgebung, Fettgehalt) fokussierten (Buvé et al., 2022). Ein Lebensmittel ist jedoch komplexer aufgebaut und das Zusammenspiel der einzelnen Komponenten sollte in der Beurteilung der Qualität eines Lebensmittels Berücksichtigung finden. Das *Knowledge Centre for Food Fraud and Quality* umschreibt Lebensmittelqualität als "[..] *a complex and multidimensional concept which is influenced by a wide range of situational and contextual factors*" (Knowledge Centre for Food Fraud and Quality, 2021). Hierbei

werden Faktoren wie die Sensorik, Authentizität, Herkunft oder auch Sicherheit aufgeführt. Aufgrund der Vielzahl an Faktoren, die die Zusammensetzung eines Lebensmittels beeinflussen können, rücken Analyseverfahren in den Vordergrund, welche simultan mehrere Komponenten erfassen können und somit große Datensätze mit vielen Informationen generieren. Um diese Datensätze auswerten zu können, werden multivariate Auswerteansätze eingesetzt. Dabei werden mathematische Methoden angewandt, die es ermöglichen, die Dimensionalität der Daten zu reduzieren und dabei wichtige Informationen/Zusammenhänge zu extrahieren (Buvé et al., 2022). Im Bereich der Speiseölauthentifizierung wurde in den letzten Jahren vermehrt die multivariate Datenanalyse angewendet, um u. a. die geografische Herkunft eines Speiseöles zu bestimmen (Zettl et al., 2017) oder um unbekannte Verfälschungsmittel in Speiseölen detektieren zu können (McDowell et al., 2019; McDowell et al., 2018). Hierbei werden je nach Fragestellung und Analysetechnik verschiedene *pre-processing*-Schritte und Modelltypen (*unsupervised* und *supervised*), aber auch verschiedene Kriterien zur Beurteilung der errechneten Modelle herangezogen (*performance* Parameter). Im Folgenden werden die in der vorliegenden Arbeit verwendeten *pre-processing*-Schritte, Modelltypen und *performance* Parameter kurz erläutert.

2.2.4.1 Pre-processing der Daten

Der erste Schritt, bevor multivariate Verfahren angewendet werden, ist die Vorverarbeitung (*pre-processing*) der originalen Messdaten. Mit Hilfe des *pre-processings* können u. a. Basislinienverschiebungen, Signalrauschen, Streueffekte korrigiert bzw. eliminiert werden, um somit ausschließlich relevante Informationen aus dem Spektrum für die anschließende Auswertung zu erhalten (Mishra et al., 2020).

Zudem können durch die Anwendung eines optimierten *pre-processings* Modellparameter (u. a. Sensitivität, Spezifität) verbessert werden (Horn et al., 2018; Martyna et al., 2020).

Je nach spektroskopischer Analysetechnik kann die Wahl des *pre-processings* variieren (Horn et al., 2018). Dies kann u. a. durch unterschiedliche Anregungs-/Schwingungsprinzipien und den daraus resultierenden Signalen der verschiedenen Techniken erklärt werden. Während in der MIR- und Raman-Spektroskopie insbesondere Mittenzentrierungen und Ableitungen angewendet werden (Engel et al., 2013; McGrath et al., 2018), werden ^{1}H-NMR-Spektren häufig aufgrund der u. a. weitaus größeren Anzahl an Datenpunkten einem *binning*, aber auch einer Basislinienkorrektur und anschließender Skalierung (häufig große Intensitätsunterschiede in den Spektren) unterzogen (Mishra et al., 2020; Smolinska et al., 2012; Sobolev et al., 2019). Es gibt eine Vielzahl an verschiedenen *pre-processing*-Schritten, deren Theorie bereits eingehend in der Literatur beschrieben wurde (Emwas et al., 2018; Emwas et al., 2013; McGrath et al., 2018; Riedl et al., 2015; Smolinska et al., 2012). In Tabelle 1 werden die Schritte tabellarisch aufgeführt, welche in der vorliegenden Dissertation verwendet wurden.

Tabelle 1: In der vorliegenden Arbeit eingesetzte *pre-processing* Schritte.

Pre-processing	**Vorteile und Beispiel**
Ableitungen	- Eliminierung von Basislinieneffekten z. B. Savitzky-Golay-Ableitung (Savitzky & Golay, 1964)
Standardisierung	- Korrektur von Streueffekten z. B. *Standard Normal Variate* (SNV) (Barnes et al., 1989; Kessler, 2008)
Skalierungen	- mathematische Gewichtung der Daten z. B. Mittenzentrierung insbesondere für MIR und Raman Daten (Engel et al., 2013; McGrath et al., 2018) ; *pareto scaling* für ^{1}H-NMR Daten (van den Berg et al., 2006)
Reduzierung Datenmenge	- Reduzierung der Datenpunkte z. B. *Binning* (Smolinska et al., 2012)

Nach dem *pre-processing* der spektralen Daten können verschiedene statistische Modelle u. a. zur Klassifizierung oder Visualisierung von Zusammenhängen im Datensatz angewendet werden, wobei hier zwischen *unsupervised* und *supervised* Modellen unterschieden wird (Oliveri & Simonetti, 2016).

2.2.4.2 Unsupervised Modelle

Anhand des berechneten *unsupervised* Modells wird versucht, Muster im Datensatz zu erkennen (Jiménez-Carvelo et al., 2019). Hierzu zählt u. a. die Hauptkomponentenanalyse *(Principal Component Analysis* - PCA). Mit Hilfe der PCA kann ein großer Datensatz mit einer Vielzahl an Variablen zu einem kleinen Datensatz reduziert werden, wobei ein Großteil der Informationen des Datensatzes erhalten bleibt

(Granato et al., 2018; Rodionova et al., 2021; Wold et al., 1987). Die Ergebnisse einer PCA werden als *principal components* (PCs), *scores* und *loadings* ausgedrückt. Höher gewichtete Informationen (größte Varianz) werden in die ersten PCs eingruppiert (PC1, PC2, PCX), wobei nicht relevante Informationen wie das Geräterauschen in letztere PCs mit niedriger Varianz verlagert werden (Bro & Smilde, 2014). Anhand der *scores* können Beziehungen zwischen den Messungen wie Trends oder Gruppierungen identifiziert werden, wohingegen die *loadings* genutzt werden können, um Beziehungen zwischen den Variablen und demnach den Einfluss der Variablen auf die PCs zu ermitteln (Rodionova et al., 2021).

Bei der PCA handelt es sich um eine Visualisierungsmöglichkeit, um einen Überblick über die Zusammenhänge der generierten Daten zu erhalten (Bro & Smilde, 2014). Insbesondere in der nicht-zielgerichteten Authentizitätsprüfung wird die PCA zur ersten Betrachtung multivariater Datensätze und auch zur Ausreißererkennung (Ehlers et al., 2022; Horn et al., 2021) eingesetzt. Obwohl vermehrt Publikationen veröffentlicht werden, die PCA-basierte Modelle als Klassifizierungsmodelle beschreiben, eignen sich diese nicht zur Klassifizierung (Vorhersage) neuer Proben. Hierfür eignen sich *supervised* Modelle.

2.2.4.3 Supervised Modelle

Diese Art der Auswertung kann als eine multivariate Klassifizierungs- oder Regressionsmethode angesehen werden, um neue Proben vorherzusagen (Bro & Smilde, 2014). Hierfür werden *supervised* Modelle angewendet, welche auf der Grundlage basieren, dass ein Datensatz mit bekannten Zielgrößen (u. a. Verfälschungsgrad oder Klassenzugehörigkeit) für die Modellentwicklung herangezogen wird (Kessler, 2008; Riedl et al., 2015). Mit dem optimierten Modell

können dann unbekannte Proben klassifiziert/vorhergesagt werden. Zu den am häufigsten angewandten Methoden in der nicht-zielgerichteten Authentizitätsprüfung gehören Modelle wie die *Partial Least Squares*-Regression (PLS-R) oder die *orthogonal projection to latent structures*-Regression (OPLS-R) als Regressionsmethoden oder die PLS-DA oder *Orthogonal Partial Least Squares* (OPLS)-DA als Klassifizierungsmethoden (z. B. zur Bestimmung der geografischen Herkunft). Erstere werden insbesondere zum Nachweis und zur Quantifizierung bestimmter Verfälschungen in Speiseölen eingesetzt (Alonso-Salces et al., 2022; Balbino et al., 2022; Haughey et al., 2015; Jiménez-Carvelo et al., 2017; McDowell et al., 2019; McDowell et al., 2018; Pfister et al., 2018; Rohman et al., 2014). Die Regressionsmodelle werden hierbei mit "verfälschten" Proben trainiert, die bekannte Konzentrationen von verschiedenen Verfälschungsmitteln erhalten.

a) Partial Least Squares-Regression (PLS-R)

Bei der PLS-R oder auch „Projektion der latenten Strukturen“ (Abdi, 2010) handelt es sich um eine multivariate Quantifizierungsmethode (Eriksson et al., 2006; Wold et al., 2001). Diese Form der Auswertung kombiniert Eigenschaften der PCA mit den Eigenschaften der multiplen, linearen Regression. Mit Hilfe der PLS-R können abhängige Variablen (z. B. Wellenzahlen) anhand von unabhängigen Variablen (z. B. der Anteil einer Verfälschung von raffiniertem Rapsöl in einer Probe von kalt gepresstem Kürbiskernöl) vorhergesagt werden. Dies wird erreicht, indem orthogonale Faktoren (latente Variablen) berechnet werden, welche die beste Vorhersagekraft beinhalten (Abdi, 2010; Wold et al., 2001). Die PLS-R wird im Bereich der Authentizitätsprüfung von Speiseölen insbesondere eingesetzt, um bekannte Verfälschungen (z. B. natives Olivenöl verfälscht mit anderen Speiseölen)

quantifizieren zu können (Jiménez-Carvelo et al., 2019; Przykaza et al., 2021; Rifna et al., 2022) (detaillierte Informationen siehe **Publikation A**).

b) Partial Least Squares-Discriminant Analysis (PLS-DA)

Die PLS-DA ist ein häufig genutztes Klassifizierungsverfahren für Datensätze, deren Variablenzahl die Probenzahl überschreitet. Da die PLS ursprünglich als Regressionsverfahren in der multivariaten Statistik eingesetzt wurde (Wold et al., 2001), verläuft die Berechnung eines PLS-DA Modells ähnlich dem eines Kalibriermodells bei der PLS-R (siehe 2.2.4.3a) (Barker & Rayens, 2003). Der Unterschied besteht jedoch darin, dass anhand verschiedener Informationen (z. B. Spektren) eine Probe einer bestimmten Klasse zugeordnet wird. Es wird hierbei von einem *hard classifier* gesprochen, da jede Probe einer Klasse zugeordnet wird (Barker & Rayens, 2003; Brereton & Lloyd, 2014; Ruiz-Perez et al., 2020). Auch die PLS-DA wird häufig verwendet, um Speiseölauthentizitätsfragestellungen zu untersuchen (Przykaza et al., 2021; Rifna et al., 2022).

Supervised Methoden werden zudem auch zur Ausreißerdetektion (Barnett & Lewis, 1984; Kordos et al., 2010) eingesetzt. Im Folgenden werden die einzelnen Modelle zur Ausreißerdetektion kurz beschrieben, die auch in der vorliegenden Dissertation Anwendung fanden.

c) Outlier score-basierte Methoden

Diese Art der *supervised* Methoden wird in der nicht-zielgerichteten Authentizitätsprüfung bislang kaum eingesetzt, um Ausreißer im Datensatz zu identifizieren. Der Vorteil gegenüber einer PCA liegt darin, dass konkrete *outlier scores*

berechnet werden, die herangezogen werden können, um zu beurteilen, wie weit ein neues Objekt (Probe) von der Referenz (bekannter Datensatz) entfernt liegt. Hierbei wird zwischen i) distanzbasierten Methoden wie *k-Nearest Neighbour* (*k*-NN) (Kordos et al., 2010) und *Euclidian Distance* (ED) (Chandola & Kumar, 2009), welche auf der Berechnung von Distanzen zwischen zwei Punkten (je nach Algorithmus unterscheiden sich diese Punkte) basieren, ii) dichte-basierten Methoden wie dem *Local Outlier Factor* (LOF) (Breunig et al., 2000), welche zur Distanz auch die Dichte der Datenpunkte im Datenraum in die Berechnung mit einbeziehen und iii) wahrscheinlichkeits-basierten Methoden wie der *Kernel Density Estimation* (KDE) (Turlach, 1993), die unabhängig von einer spezifischen Verteilung (z. B. Normalverteilung) ist, unterschieden.

2.2.4.4 Modellentwicklung, -optimierung und -validierung

Analytische Verfahren müssen validiert werden, um in der Routineanalytik und in der Lebensmittelüberwachung zuverlässig eingesetzt werden zu können. In der klassischen, zielgerichteten Analytik werden Methoden/Verfahren bereits seit Jahrzehnten validiert. Validierung wird als „Bestätigung durch Bereitstellung eines objektiven Nachweises, dass die Anforderungen für einen spezifischen beabsichtigten Gebrauch oder eine spezifische beabsichtigte Anwendung erfüllt worden sind," (ISO 9000:2015, 2015) beschrieben. Zudem gibt es für viele Anwendungsbereiche Normen, Leitfäden und Standards, die die Vorgehensweise und den Umfang von Validierungen beschreiben und als Orientierung genutzt werden können oder für die Akzeptanz der Methode beachtet werden müssen (DIN EN ISO/IEC 17025:2018-03, 2018; ISO 9000:2015, 2015; Magnusson & Örnemark, 2014). In der nicht-zielgerichteten Analytik sind solche Regelwerke jedoch noch nicht vollumfänglich etabliert, da sich die

Herangehensweisen in der Regel nach der Untersuchung einzelner Zielanalyten richten.

Die Besonderheit der nicht-zielgerichteten Analytik ist zudem, dass es neben der Validierung des Analyseverfahrens zudem notwendig ist, die eingesetzten statistischen Modelle zu validieren. Auch in diesem Bereich gibt es bisher keine einheitlichen Validierungsstrategien, die von allen Laboratorien gleichermaßen eingesetzt werden (Donarski et al., 2019; Riedl et al., 2015). Um die Zuverlässigkeit eines Modells jedoch beurteilen zu können, ist es unerlässlich, diese Modelle in der Authentizitätsprüfung zu validieren (Riedl et al., 2015). In der vorliegenden Dissertation wurde das Konzept nach Riedl *et al.* als Validierungsgrundlage genutzt (Erläuterungen siehe Kapitel 5.1), welches verschiedene Validierungsschritte beinhaltet (Abbildung 2) (Riedl et al., 2015). Zunächst wird der gesamte Datensatz durch z. B. Kennard-Stone oder andere Algorithmen (Riedl et al., 2015) in einen Trainings- und einen Testdatensatz geteilt. Anschließend wird eine interne Validierung des Trainingsdatensatzes durchgeführt, also eine Kreuzvalidierung z. B. mittels *venetian blind* Algorithmus (Ballabio & Consonni, 2013). Diese interne Validierung wird durchgeführt, um ein *under-* oder *overfitting* des Modells zu vermeiden. Ein *underfitting* tritt dann auf, wenn das Modell nicht in der Lage ist, eine Beziehung zwischen den Daten des Trainingssatzes und des Testsatzes herzustellen, was u. a. in einer geringen Varianz und einem hohen Bias resultiert. Beim *overfitting* werden beispielsweise das Rauschen oder andere zufällige Schwankungen in das Modell mit einberechnet, was zu sehr guten, überangepassten, aber nicht validen Ergebnissen führt, wenn Messungen neuer Proben mithilfe des Modells vorhergesagt werden (Bashir et al., 2020; Riedl et al., 2015). Im Anschluss an die interne Validierung auf Grundlage der Trainingsdaten erfolgt eine externe Validierung mit dem Testdatensatz.

Bei Vorliegen eines unabhängigen, unbekannten Datensatzes, kann ergänzend eine sog. *system challenge* durchgeführt werden.

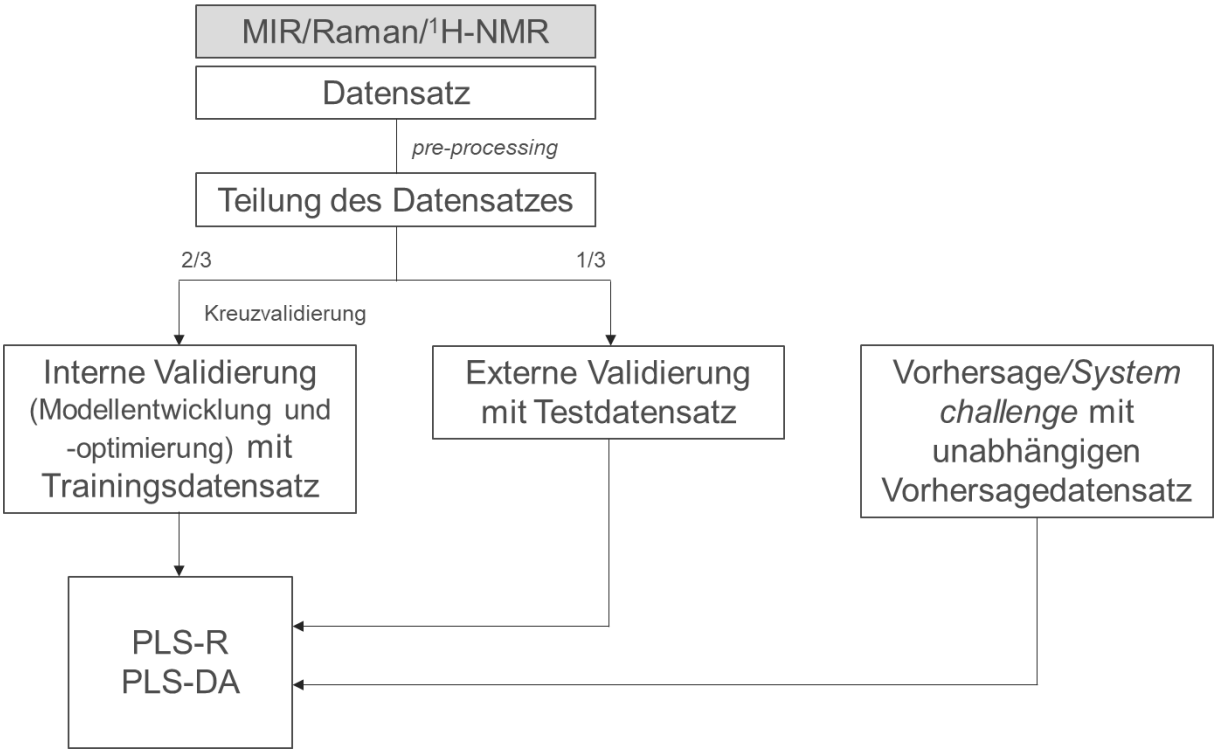

Abbildung 2: Vorgehenswese in der Modellentwicklung, -optimierung und -validierung, ^{1}H-NMR: Protonen-*Nucelar Magnetic Resonance*-Spektroskopie, MIR: *Midinfrared*-Spektroskopie, PLS-R: *Partial Least Squares-Regression*, PLS-DA: *Partial Least Squares-Discriminant Analysis*.

Je nach Klassifizierungs- und Regressionsmodell werden unterschiedliche Leistungsparameter (sog. *performance* Parameter) zur Beurteilung der Modellergebnisse herangezogen (Riedl et al., 2015). In Tabelle 2 sind die wichtigsten *performance* Parameter für ein PLS-R- (Andrade et al., 2019; Eriksson et al., 2013; Jamwal et al., 2020) und PLS-DA-Modell (López et al., 2015) aufgelistet.

Tabelle 2: Wichtige *performance* Parameter für die Beurteilung der PLS-R und PLS-DA Modelle.

PLS-R	PLS-DA
$RMSE = \sqrt{\sum_{i=1}^{n} \frac{y_i - \hat{y}_i^2}{n}}$	*Sensitivity* [%] $= \frac{TP}{TP + FN}$
$R^2 = 1 - \frac{\sum(y_i - \hat{y}_i)^2}{\sum(y_i - \bar{y})^2}$	*Specificity* [%] $= \frac{TN}{FP + TN}$
$BIAS = (\bar{\hat{y}} - \bar{y})$	*Accuracy* [%] $= \frac{TP + TN}{TP + FN + FP + TN}$
	Precision [%] $= \frac{TP}{TP + FP}$
	Classification error [%] $= \frac{(100\text{-Sensitivity}) + (100\text{-Specificity})}{2}$
mit y_i: Referenzgehalt der Probe, $\hat{y}_i$: vorhergesagter Gehalt anhand des Modells, n: Probenanzahl, $\bar{\hat{y}}$: Mittelwert des vorhergesagten Wertes, $\bar{y}$: Mittelwert des aktuellen (gemessenen) Wertes.	mit TP: *True Positive*, FP: *False Positive*, TN: *True Negative*, FN: *False Negative*.

PLS-R: *Partial Least Squares-Regression*, PLS-DA: *Partial Least Squares-Discriminant Analysis*.

In der vorliegenden Arbeit wurden diese *performance* Parameter jeweils für die interne und externe Validierung sowie für die Vorhersage mit identischen Proben (**Publikation C**) basierend auf einem PLS-DA-Modell und *system challenge* (**Publikation A**) basierend auf einem PLS-R-Modell berechnet und anhand dessen die Qualität eines Modells bewertet. Je niedriger und vergleichbarer beispielsweise die RMSE-Werte für *Calibration* (RMSEC), *Cross Validation* (RMSECV) und *Prediction* (RMSEP) sind, desto besser ist die Qualität eines Modells (Liu et al., 2017; Riedl et al., 2015).

3 Problemstellung und Zielsetzung

In der Literatur wird eine Vielzahl von Studien beschrieben, die sich mit der Verwendung von nicht-zielgerichteten Analysetechniken für die Authentifizierung von Speiseöl befassen, insbesondere mittels MIR- (Javidnia et al., 2013; McDowell et al., 2018), Raman- (Berghian-Grosan & Magdas, 2020, 2021; Jiménez-Carvelo et al., 2017; McDowell et al., 2018) und ^{1}H-NMR- (Alonso-Salces et al., 2022; McDowell et al., 2019) Spektroskopie in Kombination mit multivariater Datenanalyse. All diese Studien verfolgen ein Ziel: Die Bekämpfung von *Food Fraud* voranzutreiben bzw. Analyseverfahren zu entwickeln, die es gestatten, Fälle von *Food Fraud* frühzeitig aufzudecken. Das Ideal ist die Konzeption von Ansätzen, die nicht nur bekannte, sondern auch bisher unbekannte Praktiken von *Food Fraud* zuverlässig nachweisen können. Um dieser betrügerischen Vorgehensweise schnellstmöglich auf die Spur zu kommen, ist es notwendig, dass die Analyseverfahren ohne langwierige Probenvorbereitung auskommen und die Messung der Probe schnell durchgeführt werden kann. Daher war das **Ziel 1** der vorliegenden Arbeit Analyseverfahren zu entwickeln, mit denen Speiseölverfälschungen (erstmalig am Beispiel von nativem Kürbiskernöl verfälscht mit raffiniertem Rapsöl) ohne jegliche bzw. mit geringer Probenvorbereitung und mit einer einzigen Messung detektiert werden können. Hierbei wurden drei spektroskopische Techniken (FT-MIR, FT-Raman, ^{1}H-NMR) in Kombination mit einer multivariaten Regressionsanalyse (PLS-R) getestet (**Publikation A**). Anhand der erhaltenen Ergebnisse sowie im Hinblick auf die Schnelligkeit und Handhabbarkeit wurden die gewählten Ansätze verglichen und Vor- und Nachteile evaluiert. In der beschriebenen Literatur zum Thema nicht-zielgerichtete Analytik sind bisher keine einheitlichen Kriterien (*performance* Parameter, Validierungsstrategien) zur Beurteilung der berechneten Modelle allgemein etabliert.

Dieser notwendige Harmonisierungsaspekt wird des Weiteren in **Publikation A** herausgestellt und verschiedene Strategien diskutiert.

Neben dem Aspekt der Entwicklung einheitlicher Strategien zur Validierung von multivariaten Modellen müssen für die Anwendung der nicht-zielgerichteten Analytik zudem noch eine Reihe weiterer Harmonisierungs- bzw. Standardisierungsaspekte erfüllt werden. Dazu gehören (i) Qualitätssicherungsmaßnahmen, (ii) Gewährleistung der Vergleichbarkeit z. B. spektraler Daten und (iii) gemeinsam nutzbare Datenbanken mit einheitlichen Datenaustauschformaten.

Der Aspekt der Qualitätssicherung, wie die Verwendung einer Qualitätssicherungs-(QS)-Probe, welche messtäglich analysiert wird, findet in vielen Studien zur nicht-zielgerichteten Authentizitätsprüfung wenig Beachtung und eine einheitliche Vorgehensweise (auch bei der Auswertung) gibt es derzeit noch nicht. Bislang werden QS-Proben in die Analyse so integriert, dass bei einer meist PCA-basierten Auswertung überprüft wird, ob die Varianz der QS-Proben geringer ist als die Varianz der analysierten Proben (Achten et al., 2019; Ehlers et al., 2022; Horn et al., 2021). Wird diese Voraussetzung erfüllt, kann angenommen werden, dass das Verfahren grundsätzlich geeignet ist (Sangster et al., 2006). Diese Vorgehensweise wurde für die spektroskopische, nicht-zielgerichtete Analytik aus dem *metabolomics* Bereich adaptiert. Die Einhaltung qualitätssichernder Maßnahmen nach etablierten rechtlichen Anforderungen und relevanten Normen analog zur zielgerichteten Analytik (DIN EN ISO/IEC 17025:2018-03, 2018; DIN ISO 5725, 1994; Kommission, 2002) sowie eine einheitliche Durchführung dieser Maßnahmen in allen Laboratorien, würde es ermöglichen, nicht-zielgerichtete Verfahren gerichtsfest einsetzen zu können. **Ziel 2** war es daher, eine geeignete Auswertestrategie zur Erstellung einer multivariaten Kontrollkarte, basierend auf FT-MIR-Messungen einer raffinierten Rapsölprobe (als

QS-Probe), mit konkreten Werten beispielhaft zu entwickeln (**Publikation B**). Diese Kontrollkarte wurde in Anlehnung an die Anforderungen und Vorschriften in der zielgerichteten Analytik einer QS-Probe konzipiert.

Eine weitere Herausforderung in der Anwendung von nicht-zielgerichteten Verfahren in der Routine besteht darin, dass die bisher entwickelten Analyseverfahren stets auf individuellen Lösungen basieren. Dies bedeutet, dass oftmals jedes Labor/jede Institution eine eigene interne Datenbank aufbaut, um Spektren einer unbekannten Probe mit Referenzdaten abzugleichen. Für den Aufbau und die Nutzung einer gemeinsamen Datenbank muss jedoch die Vergleichbarkeit von spektralen Daten, welche in verschiedenen Laboratorien und mittels unterschiedlicher Geräte erfasst werden, gewährleistet werden. Erst wenn diese Datenkompatibilität hergestellt ist, können Spektren/Modelle diverser beteiligter Institutionen in einer einzigen Datenbank hinterlegt und für andere Labore zugänglich gemacht werden. Das **Ziel 3** in der vorliegenden Dissertation lag daher in der Untersuchung und dem Vergleich von Korrekturansätzen zur Gewährleistung der Vergleichbarkeit von MIR-Spektren (**Publikation C**). Dies wurde ebenfalls beispielhaft an Kürbiskern- und Rapsölproben untersucht, welche an drei verschiedenen MIR-Geräten in unterschiedlichen Laboren gemessen wurden.

4 Kumulativer Teil

Die nachfolgenden Veröffentlichungen sind Teil der kumulativen Arbeit, welche im *Peer-Review*-Verfahren begutachtet und in internationalen Fachzeitschriften veröffentlicht wurden:

4.1 *Comparison of spectroscopic techniques using the adulteration of pumpkin seed oil as example*

Publikation A - *Comparison of spectroscopic techniques using the adulteration of pumpkin seed oil as example*

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RESEARCH

Comparison of Spectroscopic Techniques Using the Adulteration of Pumpkin Seed Oil as Example

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Abstract
The aim of the present study was to compare different spectroscopic techniques using the example of adulteration of pumpkin seed oil with rapeseed oil in combination with a multivariate regression method. A total of 124 pure seed oils and 96 adulterated samples (adulteration levels from 0.5 to 90.0% w/w) were analyzed using mid infrared, Raman, and ^{1}H-nuclear magnetic resonance spectroscopy. To build quantification models, partial least squares regression (PLS-R) was used. The regression performance parameters, latent variables, and the detection limits (in terms of root mean square error of PLS prediction) calculated when applying the different spectroscopic approaches were compared. For the studied example (pumpkin seed oil adulterated with refined rapeseed oil), the lowest detection limit (3.4% w/w) was obtained for ^{1}H-nuclear magnetic resonance spectroscopy. For the mid infrared and Raman spectroscopy, detection limits of 4.8% w/w and 9.2% w/w, respectively, were obtained, which might be used as screening methods.

Keywords Spectroscopy · Chemometrics · PLS-R · Edible oil · Adulteration

Introduction

The Food Fraud Network's report, published by the European (EU) Commission, annually indicates the most frequently listed product categories in the Administrative Assistance and Cooperation (AAC) system (European Commission 2023). This system collects requests from EU member states to share information about non-compliances and potential deliberate violations of EU legislation in order to tackle food fraud. In 2022, numerous requests were attributed to the product category "fats and oils" (5th place), with the highest number of non-compliance notifications due to "mislabeling" (European Commission 2023), which could be an indication of fraudulent practices.

Cold-pressed pumpkin seed oil is a variety of oil, which is highly susceptible to adulteration with other edible oils. Compared to other seed oils (such as refined rapeseed oil), this is a high-priced oil due to the costly cultivation of the crop, the manual harvesting of the seeds, the extraction of the oil (Wenzl et al. 2002), and nutritional benefits (Šamec et al. 2022). In contrast, refined rapeseed oil is produced in a large-scale industrial process (including mechanical harvesting of the seeds), which is associated with a high oil yield, making it an inexpensive product. Furthermore, pumpkin seed oil has specific organoleptic properties (strong inherent taste as well as odor and a dark green color), which may complicate identification of neutral edible oils as adulterants. For instance, due to the refinement process, the color of a refined rapeseed oil varies from saturated light yellow to light yellow and the taste is almost neutral. Thus, the color is easily concealed when mixed with other edible oils. Since such adulterations can hardly be determined through organoleptic investigation, robust, fast, and reliable analytical methods are required for the detection and prevention of instances of food fraud (Regulation (EU) 2017/625 2017).

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With respect to the detection of adulteration in the edible oil domain, several chromatographic approaches are well established, such as gas chromatography (GC) using a flame ionization detector (FID) (Official Method 2009/7th) or high-performance liquid chromatography (HPLC) in combination with evaporative light scattering (HPLC-ELSD) detector. These techniques are applied in particular to characterize the triacylglycerol and the fatty acid profiles (Salghi et al. 2014). If different types of olive oils cannot be distinguished on the basis of their fatty acid and triacylglyceride profile, the content of minor components (e.g., sterols) as identifying features can be determined by coupling HPLC and GC (HPLC-GC) (Grob et al. 1990). Such approaches are usually the basis of targeted methods, i.e., they are specific to certain molecules or profiles. One advantage of these targeted methods involves the ability to reliably identify and calculate low limits of detection (LOD). For instance, Wenzl et al. (2002) developed a method to discover adulteration of pumpkin seed oil using GC-FID, based on the quantification of the β-sitosterol content in pumpkin seed oil (Wenzl et al. 2002). The authors determined a LOD of 3.3 mg/L and showed that the addition of 1% by weight of corn oil to genuine pumpkin seed oil increased the β-sitosterol content by about 35%. However, a major disadvantage is that most of these chromatography-based techniques involve time-consuming sample preparation, including steps such as acylation, saponification, and various derivatizations. Therefore, rapid measurement and evaluation of the sample is not possible, which hinders detecting food fraud in an early stage. In addition, hazardous substances such as *n*-hexane, methanol, or different ethers are required during these sample preparations, which are hepatotoxic or even toxic to the central nervous system and, consequently, require experienced and trained personnel. Moreover, the analytical approaches described focus solely on the investigation of targeted, i.e., known substances or compositions (based for instance on the fatty acid profile), whereas unknown mixtures, whose presence or absence may provide more specific information, might be overlooked.

To circumvent these drawbacks, non-targeted analysis has been used more frequently in recent years to authenticate food products. This analytical approach uses, depending on the analytical method used, the entire spectrum/chromatogram of a specific food matrix in combination with chemometric evaluation for a comprehensive sample characterization. Accordingly, in order to capture the so-called chemical fingerprint and to avoid elimination of typical compounds of a sample, non-selective sample preparation is required (Esslinger et al. 2014; McGrath et al. 2018). Therefore, in contrast to targeted chromatographic methods, extensive sample preparation procedures such as esterification, transesterification, or fat extraction are not necessary (Castejón et al. 2014). In addition to being more time efficient, this procedure often provides the advantage of a reduced use of hazardous chemicals.

Thus, several studies are available focusing on non-targeted screening tools using mid infrared (MIR) (Fernández Pierna et al. 2016; Javidnia et al. 2013; McDowell et al. 2018), Raman (Berghian-Grosan and Magdas 2020, 2021; Jiménez-Carvelo et al. 2017; McDowell et al. 2018), near infrared (NIR) (Baeten et al. 2014), and nuclear magnetic resonance (NMR) (Alonso-Salces et al. 2022; McDowell et al. 2019) spectroscopy in combination with multivariate data analysis.

In recent years, spectroscopy-based approaches have been employed to develop multivariate regression models, such as partial least squares-regression (PLS-R) analysis or orthogonal projection on latent structures (OPLS) regression, which can be applied to detect and quantify specific adulterants in edible oil (Alonso-Salces et al. 2022; Balbino et al. 2022; Haughey et al. 2015; Jiménez-Carvelo et al. 2017; McDowell et al. 2019; McDowell et al. 2018; Pfister et al. 2018; Rohman et al. 2014). For this purpose, the regression models are trained with "adulterated" samples containing known concentrations of different adulterants.

Rohman et al. (2014) were able to detect adulterations of canola oil in extra virgin olive oil at concentrations of 1% *v/v* using MIR spectroscopy and a PLS-R analysis (Rohman et al. 2014). In contrast, Christopoulou et al. (2004) were able to detect only 5% of canola oil in olive oil using commonly applied targeted, chromatographic methods in combination with a considerably more time-consuming sample preparation (Christopoulou et al. 2004). In another study using near-infrared spectroscopy (NIR), colorimetry, and GC-MS, in combination with OPLS regression, Balbino et al. (2022) investigated the adulteration of pumpkin seed oil with refined sunflower oil (Balbino et al. 2022). The root mean square error of estimation (RMSEE) and of cross validation (RMSECV) values obtained ranged from 2.298 to 6.668 based on different sterol contents, but the estimated LODs were not calculated. The detection of mineral oil in sunflower oil was studied by Pfister et al. (2018) using NIR and MIR spectroscopy. The LOD of 0.12% w/w for NIR and 0.16% w/w for MIR were determined based on the calculation of three times the standard deviation of the predicted mineral oil content of the non-spiked samples (Haughey et al. 2015; Pfister et al. 2018). This example illustrates that very low LOD can be obtained using spectroscopy-based and regression models.

The adulteration of cold-pressed rapeseed oil with refined sunflower and rapeseed oils using MIR and Raman spectroscopy in combination with multivariate regression analysis (PLS-R) was described by McDowell et al. (2018) (McDowell et al. 2018). The authors obtained minimum detection levels (based on 2 × RMSE of prediction (RMSEP)) of 15% (Raman) and 9% (MIR) for adulteration

with sunflower oil. In a further study, McDowell et al. (2019) compared PLS-R results from low and high-field ^{1}H-NMR spectroscopy for the detection of adulterated cold-pressed rapeseed oil with other refined edible oils (McDowell et al. 2019). Based on PLS-R results, minimum detection levels (MDLs) for the adulteration with refined sunflower oil of 8% (400 MHz low field) and 12% (60 MHz) were determined. As a result, the adulteration of cold-pressed rapeseed oil with refined sunflower oil indicated that ^{1}H-NMR spectroscopy (400 MHz) yielded the lowest minimum detection level.

Alonso-Salces et al. (2022) determined RMSEP values between 0.32 and 3.4 (% vegetable oil) for adulteration of olive oil with different oil varieties based on ^{1}H-NMR spectroscopy (500 MHz) and statistical data analysis (Alonso-Salces et al. 2022). These publications highlight strong differences, not only regarding the approach to data evaluation, such as model development and optimization, but also in the assessment of the developed models.

Therefore, the aim of the presented study was to evaluate and compare the potential of three spectroscopic methods (MIR, Raman, and ^{1}H-NMR spectroscopy) as screening tools using the results of multivariate, quantitative regression analysis to investigate the adulteration of cold-pressed pumpkin seed oil with refined rapeseed oil.

Materials and Methods

Sample Collection and Preparation

For this study, 44 rapeseed and 80 pumpkin seed oil samples, purchased from the German retail market from 2017 to 2019, were randomly analyzed within each seed edible oil group. Variability among each seed oil group was covered as best as possible by purchasing oils from different manufacturers, batch numbers, production processes, etc. (Table S1 in the Supplementary material).

The following procedure describes the preparation/selection of the different sample types:

(a) Pooling of pumpkin seed oil samples

In order to cover the highest variability of the pumpkin seed oil class in a sample, the 80 investigated pumpkin seed oil samples were randomly divided into four groups of 20 seed oil samples each, resulting in four pool samples. For each pool sample, 3 ± 0.02 g of the respective pumpkin seed oil samples was weighed into a 100 mL amber DURAN® bottle (Schott AG, Mainz, Germany).

(b) Rapeseed oil samples as adulterants

To identify a representative subset of the rapeseed oil class for the adulteration of the pooled pumpkin seed oil samples, principal component analysis (PCA) of the MIR data of the 44 individual rapeseed oil samples was performed. To ensure high variability within the adulterants (rapeseed oil group), four rapeseed oil samples were selected as adulterants based on the first two principal components (furthest apart from each other in the PCA scores plot) (results not shown).

(c) Adulterated samples

Each pumpkin seed oil pool sample (pool 1-pool 4) was spiked with each rapeseed oil sample (rapeseed oil 1-4), respectively. Pool samples 1 and 2 were adulterated at 19 different concentrations ranging from 0.5 to 90% w/w, and pool samples 3 and 4 were adulterated at five different concentrations ranging from 1 to 10% w/w under gravimetric control (Genius ME254S, Sartorius AG, Göttingen, Germany) (Table S2/S3 in the Supplementary material). The samples were homogenized for 3 min with a Vortex Mixer (Grant Instruments, Cambridge, UK).

The aliquots of the pure rapeseed and pumpkin seed oil samples and pool samples as well as the 96 different adulterated oil samples were filled in 1.2 mL cryogenic tubes (neoLab Migge GmbH, Heidelberg, Germany) and were finally stored under completely dark conditions at −18 °C excluding any headspace volume in order to better preserve the oil samples from oxidation.

Prior to spectroscopic analysis, the samples were tempered to 21 °C and homogenized with an overhead shaker (Reax 2, Heidolph Instruments, Schwabach, Germany) for 30 s at 40 rounds per minute.

MIR Spectroscopy

The mid infrared spectra were recorded on a Vertex 70v Fourier transform spectrometer (Bruker Corporation, Ettlingen, Germany), which was equipped with the standard air-cooled source, a single attenuated total reflectance (ATR) diamond crystal, a wideband IR beamsplitter, and a room temperature deuterated lanthanum α-alanine-doped triglycine sulfate (DLaTGS) detector. For each measurement, 1 µL of sample was transferred onto the crystal surface. Spectra for each sample were recorded in triplicate at room temperature in the absorbance mode from 3996 to 550 cm^{-1} with a spectral resolution of 4 cm^{-1} (data spacing of 1.928 cm^{-1}, Happ-Genzel apodization) by accumulating 32 scans. A background spectrum (laboratory air) was recorded immediately before each sample measurement and inspected visually in order to exclude any signals from solvent (from cleaning) or sample residues. The performance (spectral resolution, signal-to-noise-ratio, and wavenumber accuracy) of the spectrometer

was inspected every 2 months using a polystyrene standard. Spectra visualization was performed with the OPUS 6.5 software package (Bruker, Waltham, USA). The triplicate spectra were averaged and the mean spectrum was used for chemometric analysis.

Raman Spectroscopy

The Raman spectra were acquired on a Vertex 70v Fourier transform spectrometer equipped with RAM II module (Bruker Optics, Ettlingen, Germany) with a spectral range of 4000–50 cm^{-1} and a spectral resolution of 4 cm^{-1} by collecting 128 scans. The Raman module was equipped with a Nd:YAG laser source (yttrium aluminum garnet crystal doped with triply ionized neodymium) (1064 nm), a CaF_2 beamsplitter, and a liquid nitrogen cooled germanium diode detector. Each sample was placed in a glass cuvette (10 mm) and then measured in duplicate at room temperature. The performance of the spectrometer was checked every month using a polystyrene and a naphthalene standard. Visualization of spectra was performed using the OPUS 6.5 software package (Bruker Optics, Ettlingen, Germany). The duplicate spectra were averaged and the mean spectrum was used for chemometric analysis.

^{1}H-NMR Spectroscopy

All chemicals used for the ^{1}H-NMR analysis are presented in Table S4 in the Supplementary material. The measurements were performed with a 400 MHz spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) applying the instrument specifications in Table S5 in the Supplementary material.

For data collection, 140 ± 2 mg of oil was weighed into a sample tube (Safe-Lock tube, 2 mL, Eppendorf AG, Hamburg, Germany). Using a positive displacement pipette (Microman M1000, 100 µL, Gilson Inc., Middleton, USA), 700 µL of deuterated chloroform ($CDCl_3$, with 0.03% tetramethylsilane (TMS) as internal standard) was added to the same 2 mL sample tube. The weight of the sample and the added $CDCl_3$ were recorded on an analytical balance (Genius ME254S, Sartorius AG, Göttingen, Germany). The weight of the $CDCl_3$ was converted to volume (ρ = 1.5 g/cm^3). The total volume of each diluted oil sample was 700 ± 20 µL. The sample tube was sealed and the solution homogenized for 10 s on a Vortex Mixer (Grant Instruments, Cambridge, UK). From this homogenized solution, 600 µL was transferred to a 5 mm NMR tube (507-PP-7, Wilmad-LabGlass, Sigma Aldrich, St. Louis, USA) using a piston-stroke pipette. During the measurement, the pumpkin seed oil and adulterated samples were positioned in the autosampler holder (BACS-120, Bruker BioSpin GmbH, Rheinstetten, Germany) under dark conditions at room temperature to avoid oxidation processes.

All ^{1}H-NMR measurements were performed without rotation in an automated mode. After sample transfer to the magnet by the autosampler and an equilibration time of 5 min for the temperature, the following optimization steps of the NMR parameters (Godelmann et al. 2013) for each sample were carried out: (i) locking, (ii) automatic tuning and matching, (iii) shimming, and (iv) pulse calibration. Sample acquisition and processing were performed within a single experiment. The instrument settings are presented in Table S6 in the Supplementary material. The collected free induction decay (FID) was automatically processed in TopSpin (Bruker BioSpin GmbH, Rheinstetten, Germany), which included the multiplication with an exponential line broadening (LB) function of 0.3 Hz, Fourier transformation, phase and baseline (polynomial) correction. The chemical shift axis of the spectra was referenced to the signal of TMS at δ 0.00 ppm. The obtained spectra were used for multivariate data analysis.

Quality Control Sample

A refined rapeseed oil purchased from the local market (Germany) was used as quality control (QC) sample for all analysis. The QC sample was analyzed on each measurement day using the three selected spectroscopy approaches (at the beginning, the middle, and the end of each batch) and was filled and stored in the same way as described in "Sample Collection and Preparation".

In addition to the QC sample, two NMR tubes (5 mm) containing methanol-d_4 and sucrose standard solution (Bruker GmbH, Rheinstetten, Germany) were analyzed for defined parameters (absolute temperature of methanol-d_4; water suppression: length of 90 °C pulse, half-width of TMS signal, and signal-to-noise ratio) before starting the NMR measurements each working day and the corresponding assessment limits are listed in Table S7 in the Supplementary material. Whenever the half-width of the standard signal of TMS (~ 0.5 Hz, maximum 0.7 Hz at LB = 0) was >0.5 Hz, the measurements were repeated.

Multivariate Data Analysis

The pre-processing (only bucketing) of ^{1}H-NMR spectra was performed using Mnova version 12.0 (Mestrelab Research, S.L., USA). Multivariate data analysis was carried out using PLS toolbox version 7.0.3 (Eigenvector Research, Wenatchee, WA, USA) together with Matlab version 7.11.0584 R2010b (The MathWorks Inc., Natick, MA, USA).

Data Reduction and Optimal Pre-processing

Regions of the averaged spectra of MIR measurements that did not contain relevant spectral information were excluded (4000–3040 cm^{-1} and 1625–1490 cm^{-1} baseline area, 2790–1790 cm^{-1} absorption of diamond crystal). After this reduction process, each MIR spectrum consisted of 678 data points. The same procedure was performed for the Raman spectra, where the regions 4000–3100 cm^{-1}, 2600–1800 cm^{-1}, and 600–50 cm^{-1} were not included in the data analysis, resulting in 3111 data points per spectrum.

The generated Fourier transformed, phase- and baseline-corrected ^{1}H-NMR spectra were further processed. Bucketing was performed within 0.50–10.02 ppm using a bucket width of 0.04 ppm. The regions of the residues of the undeuterated chloroform signal (7.22–7.34 ppm) and without relevant information (9.02–10.02 ppm) were eliminated, leaving 203 buckets for multivariate statistical analysis.

For each spectroscopic method, PLS-R models were built, and the optimal pre-processing was identified based on the following parameters: the root mean square error of calibration (RMSEC), cross validation (RMSECV), and prediction (RMSEP) for external validation, determination coefficient (R^2) of calibration, R^2 of cross validation, R^2 of prediction as well as the number of latent variables (LV). To select the optimal number of LV, the values for RMSEC and RMESCV were plotted against the number of LVs and selecting the first minimum. Based on these parameters, different pre-processing steps and combinations (Table S8–S10 in the Supplementary material) were tested for each method, and thus, the optimal approach determined was applied: (i) MIR spectroscopy - standard normal variate (SNV), first Savitzky-Golay derivative (filter width 15; polynomial 2), Savitzky-Golay smoothing and mean center, (ii) Raman spectroscopy - SNV, first Savitzky-Golay derivative (filter width 15; polynomial 2) and mean center, (iii) ^{1}H-NMR spectroscopy - normalization (α-signals of glycerol 3.9–4.56 ppm) (Fauhl-Hassek et al. 2000) and Pareto scaling (Table S11 in the Supplementary material).

Explorative Data Analysis

Principal component analysis (PCA) was performed to reduce dimensionality by calculating principal components (PCs) (Wold et al. 1987) and to visualize the possible grouping of pure and adulterated samples, applying the singular value decomposition (SVD) algorithm. For the detection of potential outliers, Student's t distribution, Hotelling's T^2 probability distribution in combination with the Q-statistics, was used (Hotelling 1992; Joe Qin 2003).

Model Building, Optimization, and Validation Using PLS

Detection of adulterants was performed using PLS-R (Eriksson et al. 2006). This involves calculating various statistical parameters such as RMSEs and regression coefficients, which are used to assess the quality of the model (Medina et al. 2019; Riedl et al. 2015; Uncu and Ozen 2015). For this study, the RMSEC, RMSECV, and RMSEP as representation of the spectral differences between the predictions of calibration/validation steps (Liu et al. 2017; Riedl et al. 2015) as well as the determination coefficient (R^2) (Uncu and Ozen 2015) were used. Furthermore, the RMSEP of the system challenge was applied to estimate minimum detection level as shown by Downey and Kelly (Downey and Kelly 2004).

To perform a comparison of PLS-R model results, the following procedure was selected (Fig. 1) in accordance to recommendations of Riedl et al. (2015) (Riedl et al. 2015). The whole data set (pure pumpkin seed oil samples; rapeseed oil samples - except the four rapeseed oil samples used for adulteration; pool 1 and pool 3 samples, adulterated with rapeseed oil samples 3 and 4) was divided into training (66%) and test set (34%) using the Kennard-Stone (KS) algorithm (Kennard and Stone 1969) to avoid the bias that results from manual data splitting. The training set was used to build and optimize the PLS-R model. An internal cross validation was applied with ten data splits on the training set using the venetian blind algorithm (Ballabio and Consonni 2013). The test set was used for external validation to validate the PLS-R model.

Finally, the data of pool 2 and pool 4 adulterated with rapeseed oil samples 1 and 2 were included in the optimized model as independent extra test sets (so called system challenge according to Riedl et al. 2015) for prediction.

Results and Discussion

Visualization and Explorative Data Analysis

To identify differences in the composition of the two types of seed oil varieties, the respective spectral characteristics of the oil samples for each method were firstly described and an exploratory data analysis was performed. A detailed explanation of the respective fatty acid composition of the two seed oil varieties has already been described by several research groups (Baeten et al. 1998; Baeten et al. 1996; Berghian-Grosan and Magdas 2020; Castejón et al. 2014; Guillén and Cabo 1999, 2000) and therefore was not the focus of this study.

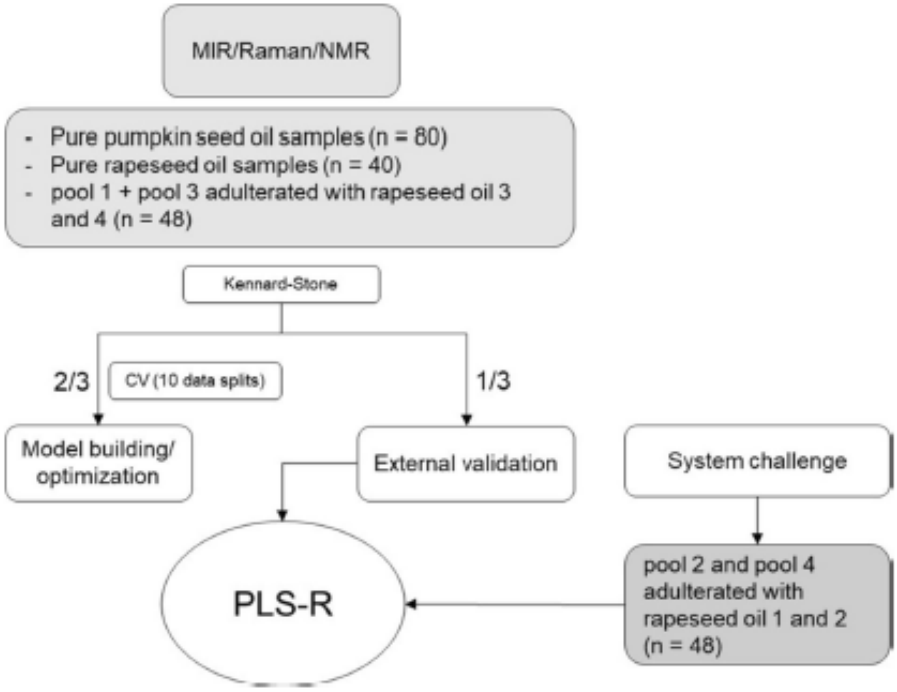

Fig. 1 Scheme of PLS-R model building/optimization, internal and external validation, and system challenge with pure seed oil and adulterated samples. CV: internal cross validation (10 data splits on training set)

MIR Spectroscopy

Figure 2a illustrates the superimposed, raw MIR (after Fourier transformation) spectra of ten cold-pressed pumpkin seed oil samples (m = 3, gray lines) and ten refined rapeseed oil samples (m = 3, black lines). The highlighted areas (Fig. 2a, regions 1 and 2) represent wavenumber regions that exhibit the most noticeable differences in absorbance between the seed oil varieties. The most prominent difference is that the spectra of the pumpkin seed oil samples exhibit higher absorbances in the range between 1050 and 880 cm^{-1} than the rapeseed oil samples. This area is associated with the content of saturated fatty acids (Sherazi et al. 2009). Pumpkin seed oil generally contains 16-19% and rapeseed oil 5-10% saturated fatty acids (Potočnik et al. 2016; Stevenson et al. 2007), which is in good agreement with the superimposed spectra.

To visualize and identify differences and similarities in the data set, a PCA was performed using the pure oil sample set and the training set. Two main groupings can be recognized in Fig. 2b, which are described in particular along PC1 (65% explained variance)—one group builds the data points of the pure rapeseed oil samples (black diamonds) in quadrants 3 and 4. The other cluster consists of the data points of pure pumpkin seed oil samples (gray squares) and adulterated samples (green and yellow triangles), with no clear separation between these to sample sets. According to the loading plot (Fig. 2c), the absorbance differences of the two seed oil varieties along PC1 are described by the range of 1050–880 cm^{-1}, which seems to correlate with the information from the line plot and thus because of the different proportion of saturated fatty acids in seed varieties. Other areas that showed differences in the two oil types in the loading plot of PC1, but barely in the visualization of the spectral data, were the bands around 2924 cm^{-1} and 2854 cm^{-1} as well as around 1746 cm^{-1}. According to literature, these bands are associated with the asymmetric and symmetric stretching vibration of the aliphatic -CH_2 functional group (different fatty acid composition of the seed oil samples and therefore –CH_2 chain lengths) and the functional group of the ester compound C=O (stretching vibration) (Guillén and Cabo 1999, 2000; Vlachos and Arvanitoyannis 2008).

Raman Spectroscopy

The raw Raman (after Fourier transformation) spectral data were not suitable for showing spectral differences (results not shown), because of background noise. Therefore, the pre-processed spectral data are presented in Fig. 3a. The areas with the clearest differences in the spectra are depicted as magnifications.

The region between 3100 and 2800 cm^{-1} reveals differences and reflects characteristic scattered bands of symmetric and antisymmetric (ν(C-H)) vibrations of the terminal chains of methyl (CH_3) and methylene (CH_2) groups of aliphatic molecules (Baeten et al. 1998). The two seed oils' different fatty acid compositions, and thus the different -CH_2 chain lengths in the oil mixtures, are probably responsible

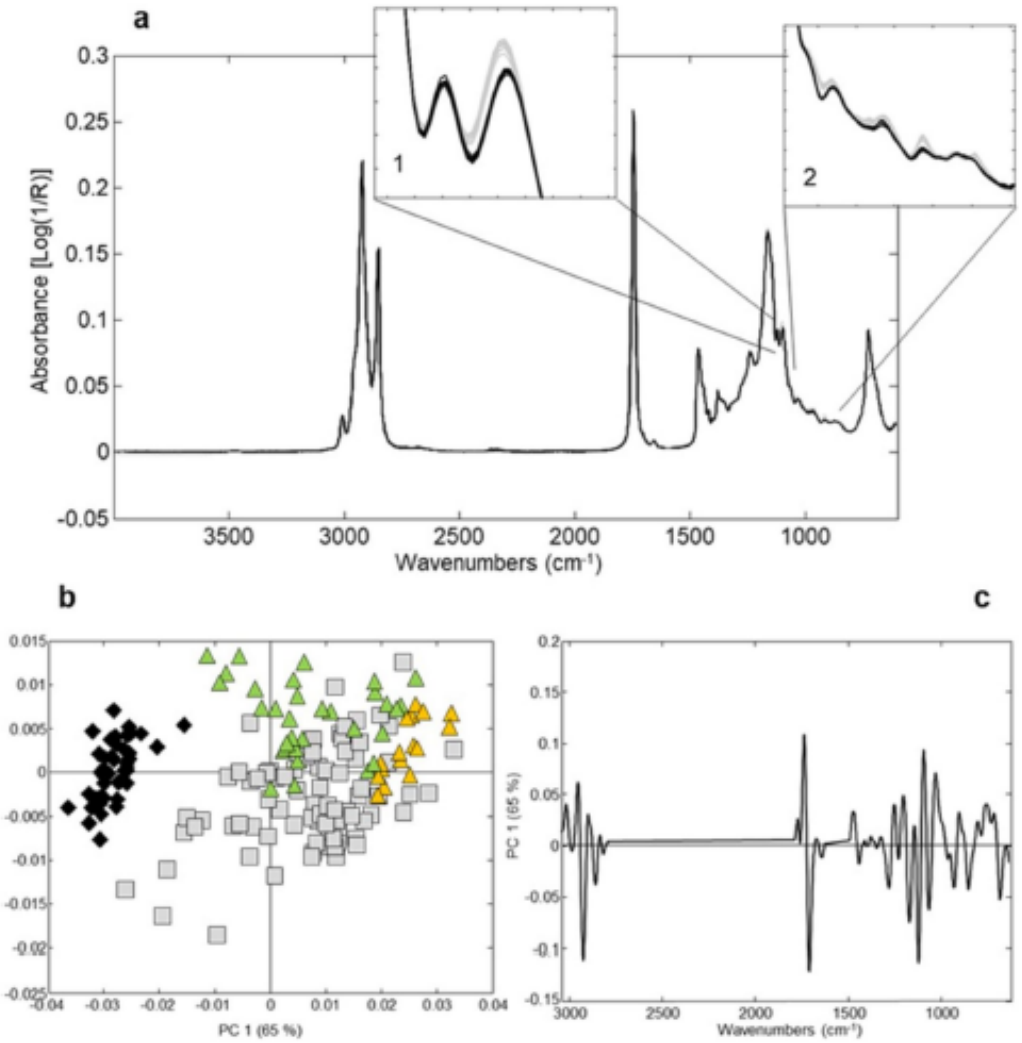

Fig. 2 **a** Line plot of MIR measurements, pumpkin seed oil samples (n = 10, gray lines), rapeseed oil samples (n = 10, black lines)—region 1/2: around 1050–880 cm^{-1}. **b** PCA score plot (PC1/PC2) of pure oil samples (rapeseed oil samples, n = 44, black diamonds; pumpkin seed oil samples, n = 80, light gray squares) and training set (n = 48, yellow triangles < 10% adulteration and green triangles > 10% adulteration) after pre-processing. **c** Loading plot of PC1

for these variations. Vibrations of the olefin groups, i.e., the saturated fatty acids, are characteristic at a Raman shift around 1660 cm^{-1} (region 2 in Fig. 3a) (Baeten et al. 1998). This also correlates with the content of saturated fatty acids in the two varieties of seed oils.

In Fig. 3a in region 3 at 1530 cm^{-1}, the spectra of the pumpkin seed oil samples (gray lines) show significantly higher scattering intensities compared to the spectra of the rapeseed oil samples (black lines). Baeten et al. (2001) demonstrated, depending on the refining process, that pigments (chlorophylls and carotenoids) were removed in edible oils that have a high intensity in this wavelength region (Baeten et al. 2001). Hence, the less extensive a refining process is, the more of these compounds remain in the edible oil and exhibit high intensities in this region of the spectrum. And in the presented study, exclusively refined rapeseed oil samples were investigated.

The apparent differences in the spectra are also described in the scores plot or by the loadings plot of the PCA. The loading plot (Fig. 3c) reveals that the grouping of the two seed oil types along PC1 is especially due to Raman scattering intensity differences in the range of 3100–2800 cm^{-1}, around 1660 cm^{-1} as well as 1050–880 cm^{-1}. Two main groupings can be observed in the PCA score plot (Fig. 3b) of the first components, discriminating along PC1 with 55% of the variance explained. This represents the seed

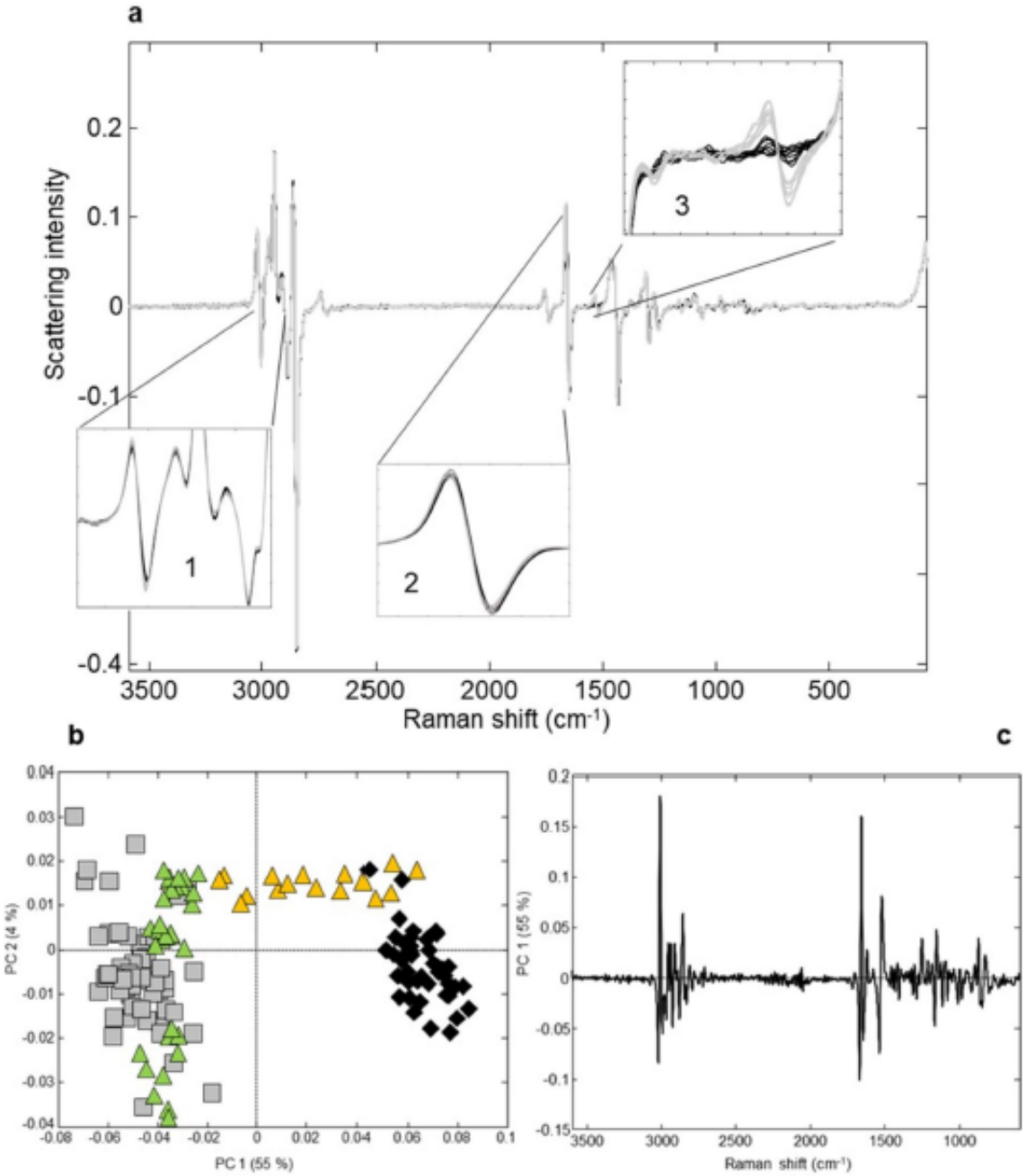

Fig. 3 **a** Line plot of pre-processed Raman spectra, pumpkin seed oil samples (n = 10, gray lines), rapeseed oil samples (n = 10, black lines) - region 1: 3100 cm–2800 cm^{-1}; region 2: 1660 cm^{-1}; region 3: 1530 cm^{-1}. **b** PCA score plot (PC1/PC2) of pure oil samples (rapeseed oil samples. n = 44, black diamonds; pumpkin seed oil samples, n = 80, light gray squares) and training set (n = 48, yellow triangles > 10% adulteration and green triangles < 10% adulteration) after pre-processing. **c** Loading plot of PC1

oil differentiation. The data points of the pure rapeseed oil samples (black diamonds) are located in quadrants 3 and 4, whereas the data points of the pure pumpkin seed oil samples (gray squares) are sited in quadrants 1 and 2. The data points from a degree of adulteration below 10% group along PC2 in the pumpkin seed oil group. The PC2 describes the variation within a seed oil group, which might be explained by different chemical compositions (due to phenotype,

harvest year, etc.) (Stevenson et al. 2007; Szydłowska-Czerniak et al. 2010).

^{1}H-NMR Spectroscopy

Figure 4a inset 1 shows ten overlaid ^{1}H-NMR spectra per pure seed oil variety. Three main spectral differences can be identified and are highlighted in the figure. In the range of 2.74–2.76 ppm, for the rapeseed oil samples (black lines), an overlap of two triplets can be identified and for the pumpkin seed oil group (gray lines) one triplet is exhibited. In this region, the signals are associated with specific bis-allylic protons (=HC-CH_2-CH=), which are also defined as poly-unsaturated fatty acids. Depending on the content and composition of the alkyl groups in the acyl group in an oil, the signals lead to different shapes in the spectrum. Oil varieties

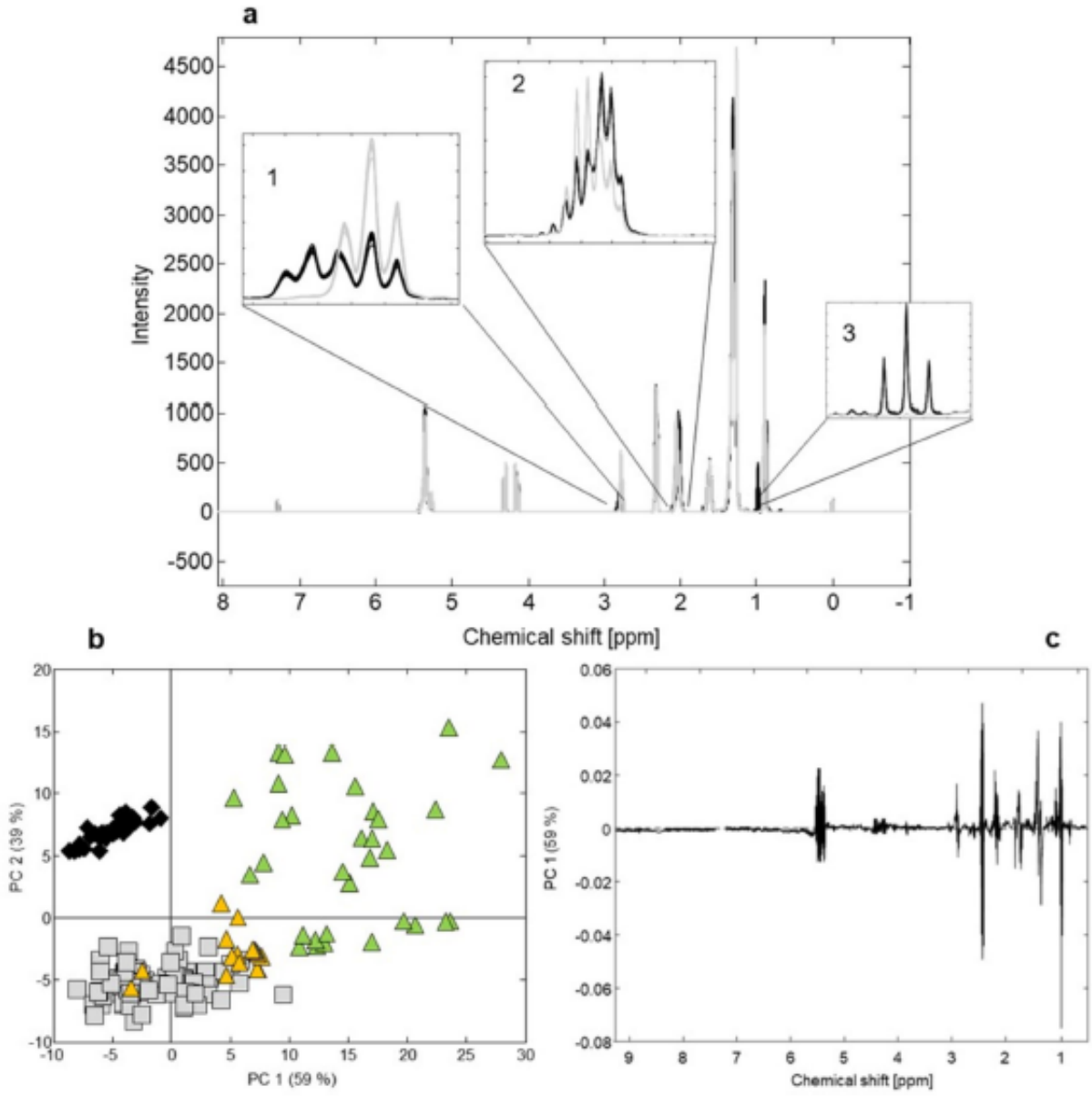

Fig. 4 **a** Line plot of ^{1}H-NMR measurements, pumpkin seed oil samples (n = 10, gray lines), rapeseed oil samples (n = 10, black lines) - region 1: 2.74–2.76 ppm; region 2: 1.99–2.01 ppm; region 3: 0.98 ppm. **b** PCA score plot (PC1/PC2) of pure oil samples (rapeseed oil samples, n = 44, black diamonds; pumpkin seed oil samples, n = 80, light gray squares) and training set (n = 48, yellow triangles < 10% adulteration and green triangles > 10% adulteration) after pre-processing. **c** Loading plot of PC1

with a high content of linoleic and linolenic acids show an overlap of two triplets. If only linoleic acyl groups and no linolenic acyl groups are present in an oil sample, the spectrum exhibits a triplet (Guillén and Ruiz 2003b).

Another difference in the oil varieties can be seen in inset 2 (1.99–2.01 ppm). Here, the shape of the signals caused by the different contents of acyl groups varies in particular. The pattern of signals differs between the two varieties. This spectral region represents also the amount and distribution of unsaturated fatty acids (specific allylic protons) (Guillén and Ruiz 2003b). In particular, the pattern for the pumpkin seed oil group at 1.99–2.01 ppm indicates (Fig. 4a, inset 2) that only small amounts of oil acyl groups are present in the samples. In contrast, the distribution of the peaks in the signal of the rapeseed oil samples implies high contents of oleaginous allyl protons in combination with smaller proportions of linoleic and linolenic acid acyl groups (Guillén and Ruiz 2003b). This correlates with the content of these fatty acids in rapeseed and pumpkin seed oil samples. While the highest content of acyl groups in rapeseed is the oleic acid groups (63%), the linoleic acid groups are dominant in pumpkin seed oils (54%) (Belitz et al. 2009; Guillén and Ruiz 2003b).

Additional variation as displayed in the line plot is present around 0.98 ppm. In this region, all rapeseed oil samples (black lines) show a triplet, whereas the pumpkin seed oil samples (gray lines) exhibit no intensity at all. This region is associated with the terminal methyl protons of *n*-3 polyunsaturated fatty acids (called Ω-3 including, e.g., linolenic fatty acid) (Castejón et al. 2014; Guillén and Ruiz 2003a). According to literature, the content of these fatty acids is 9% for rapeseed oils and only 0.5% for pumpkin seed oils (Guillén and Ruiz 2003b; Rezig et al. 2012; Sakhno 2010; Stevenson et al. 2007) explaining the different behavior in the spectra.

To consider the impact of these regions on the multivariate data analysis, a PCA was calculated. The score plot of the first two principal components is depicted in Fig. 4b. Two main groupings can be observed, differing along PC2 with 39% of the variance explained, representing the variety of seed oil samples. Along PC1 (59% of the explained variance), the variance is described within a seed oil group. The data points with a degree of adulteration of less than 10% (green triangles) scatter along PC1 and PC2 in the first quadrant. The loading plots indicate that the differences within the seed oil cluster (Fig. 4c) mostly result from the signal around 0.98 ppm, while the grouping along PC2 (results not shown) is due to differences in intensity in the range at 2.74–2.76 ppm (region 1) and 1.99–2.01 ppm (region 2).

Comparison of the Performances of the Three Spectroscopy-Based Techniques

Assessment Based on Partial Least Squares-Regression (PLS-R) Results

A common method for quantifying adulteration using spectroscopic techniques is the multivariate PLS-R. The aim of PLS is to establish the functional relationship between an independently measurable variable (e.g., wavenumbers) and a dependent target variable (e.g., levels of adulteration of refined rapeseed oil in a sample of cold-pressed pumpkin seed oil). A PLS-R was performed to estimate the MDL (2 × RMSEP) for quantifying refined rapeseed oil in cold-pressed pumpkin seed oil for each analytical technique using an independent extra test set (system challenge), according to the description of Downey and Kelly (2004). In the present study, besides the calculation of the MDL, additional focus was on the assessment and comparison of other model parameters such as RMSE and R^2 values, generated from the three spectroscopic methods. The PLS-R quantification results are summarized in Table 1.

The RMSE values (a measure of the spectral differences between the predictions of calibration/validation steps) and determination coefficients (R^2_{Pred}) were used to assess the quality of the model (Medina et al. 2019; Riedl et al. 2015; Uncu and Ozen 2015). The lower and more comparable RMSEC, RMSECV, and RMSEP values are, the more reliable is the model (Liu et al. 2017; Riedl et al. 2015). R^2_{Pred} indicates how accurately the model predicts new samples (separate independent test sets for system challenge: pool 2

Table 1 PLS-R results of MIR, Raman, and ^{1}H-NMR spectroscopy to detect refined rapeseed oil in pumpkin seed oil

Spectroscopic method	Optimal number of LV	R^2_{Pred}	RMSEC	RMSECV	RMSEP (system challenge)	Estimated MDL [% w/w]
MIR	4	0.993	2.196	3.122	2.384	4.8
Raman	3	0.983	3.202	4.465	4.591	9.2
^{1}H-NMR	3	0.998	1.148	1.326	1.722	3.4

LV optimal number of latent variables, R^2_{Pred} determination coefficient for prediction, *RMSEC* root mean square error of calibration, *RMSECV* root mean square error of cross validation, *RMSEP* root mean square error of prediction for system challenge, *MDL* estimated minimum detection level = according to Downey and Kelly (2 × RMSEP) (Downey & Kelly, 2004)

and pool 4, adulterated with rapeseed oil samples 1 and 2, see Fig. 1). In general, a R^2_{Pred} value above 0.9 also indicates high prediction ability (Uncu and Ozen 2015).

As reported in Table 1, the RMSEP and (accordingly) MDL values for Raman spectra are higher compared to the results of the MIR and ^{1}H-NMR analysis. Only for adulteration levels higher than 9.2% w/w refined rapeseed oil could be detected in pumpkin seed oil. In addition, the R^2_{Pred} value for Raman spectroscopy indicates a lower predictive ability compared to calculations based on MIR and ^{1}H-NMR spectral data. The lowest estimated MDL is at 3.4% w/w using ^{1}H-NMR spectroscopy. The highest R^2_{Pred} value was also determined for ^{1}H-NMR spectroscopy.

The different PLS-R results obtained could be explained by the different excitation/vibration principles and resulting oscillations/signals of the three molecular spectroscopic techniques. This means that when molecules are excited in different ways, depending on the type of instrument, certain vibrations from molecules can be detected differently, resulting in a difference of the information provided by the spectra. In MIR spectroscopy, molecular vibrations are excited (by, e.g., tungsten source) as a function of vibrating masses and bond strength and molecules are IR active only if they have a high dipole moment (Skoog and Leary 1996). Conjugated double bonds (e.g., linoleic acid), on the other hand, show a low dipole moment and are therefore IR inactive.

However, because Raman spectroscopy is based on a different type of excitation (by monochromatic laser beam), these bonds are Raman active and therefore can be identified in the Raman spectrum (Skoog and Leary 1996). Thus, the two analytical techniques have a complementary detection capability. In addition to the determination of the fatty acid composition, pigments or volatile components can be identified by Raman spectroscopy (see " Raman Spectroscopy") (not by MIR spectroscopy). Nevertheless, in the present study, lower detection levels of adulteration were obtained for Raman spectroscopy. Possibly, this additional information has less impact on the model results.

^{1}H-NMR spectroscopy involves the excitation of hydrogen nuclei. This allows the deduction of the electronic structure of a molecule and its functional groups, rendering this analytical technique very sensitive (Webb 2006). Depending on the shape of a signal (of a singlet, triplet, etc.) (see "^{1}H-NMR Spectroscopy") in the edible oil spectrum, individual alkyl groups in the acyl group can also be identified. In the area of *n*-3-unsaturated fatty acids, the loading plot of PC1 shows strong differences between the two oil types (see "^{1}H-NMR Spectroscopy"), which could affect the model results and consequently would explain the lower detection limits of rapeseed oil in pumpkin seed oil for ^{1}H-NMR spectroscopy. In summary, depending on the variety and production process, different chemical compounds are predominant in the edible oil. Depending on the spectroscopic technique, this results in different bands/signals in the spectrum and possibly in a different weighting of the PLS-R results.

^{1}H-NMR spectroscopy exhibits the best results (regarding PLS-R parameters) for the example reported in this study, due to its excitation principle-based sensitivity for certain molecules, which are relevant to the model, as mentioned above in "Visualization and Explorative Data Analysis."

There are no studies describing the detection of adulteration of pumpkin seed oil with refined rapeseed oil using spectroscopic techniques. However, in order to be able to classify and assess the PLS-R results, the following section measures the results with approaches described in the literature (calculations, assessment parameters, model generation) for comparable authenticity problems and spectroscopic techniques.

Balbino et al. (2022) investigated the adulteration of pumpkin seed oil with refined sunflower oil (Balbino et al. 2022). The authors used OPLS regression in combination with GC-FID and NIR spectroscopy. Differences in sterol contents between the two types of oil were observed (C-H and O-H third overtones and ArC-H and C-H first overtones). Pumpkin seed oils are rich in Δ7-sterols (except Δ7-stigmasterol), whereas refined sunflower oil has a high content of Δ5-sterols (except campestenol). The RMSEP values were not evaluated, but the authors calculated the RMSEE and RMSECV, with values of 6.470 and 6.668 determined for the model based on the β-sitosterol content and 2.298 and 2.792 based on the Δ7,22,25-stigmasterol content. These RMSECV values are within the range of those determined for the present example and indicate that the quality of the generated models is comparable. In the present study, slight differences in sterol composition (e.g., bands around 1440 cm^{-1} and 1350 cm^{-1}) (Baeten et al. 2001) between the cold-pressed pumpkin seed oil and the refined rapeseed oil samples were detected by Raman spectroscopy, but these bands took a subordinate role in the multivariate data analysis. However, by using MIR spectroscopy in our study, no specific bands for the sterol molecules could be identified from the spectra, possibly related to the fact that these molecules were not absorbed or overlapped by other bands. Raman and MIR spectroscopy can be used to obtain sample structural information (e.g., fatty acid composition), while NIR spectroscopy can be used to determine broad bands (Eliaerts et al. 2020). In another study, the adulteration of pumpkin seed oil with sunflower oil was investigated using Raman spectroscopy (portable device) (Becze and Simedru 2020). The areas of the spectral bands from the Raman spectrum were used to quantify the adulteration, and a prediction equation was developed using PLS with four band areas included. These bands were assigned to vibration bands of *cis* (C=C) and *cis* (=C-H) of unsaturated fatty acids as well as scissoring vibrations and twisting vibrations of methylene. The highest band areas in pumpkin seed oil were

assigned to the vibrations of the methylene groups while in sunflower oil the highest band area could be assigned to the vibrations of unsaturated fatty acid group. Also, in the present study, these ranges showed differences between the oil types. Nevertheless, the Raman region between 3100 and 2800 cm^{-1}, which indicated the biggest difference between the pumpkin seed oil group and the rapeseed oil group in the current study, was not included in the multivariate data analysis in the study of Becze et al. (2020) (Becze and Simedru 2020).

Further comparisons cannot be carried out due to the different model design. Pfister et al. (2018) investigated the adulteration of sunflower oil with mineral oil by NIR and MIR (Pfister et al. 2018). The authors determined the LOD values, which are based on the determination of the detection limit using the blank method. This means that in a certain number of samples that were measured the analyte they were looking for was not present (the unspiked natural sunflower oils). Using a previously generated calibration function (using multivariate PLS regression from samples spiked with mineral oil), the content of the blank samples was determined. These calculated contents were plotted in the form of a distribution diagram. From the Gaussian normal distribution fitted to the data, the detection limit was estimated using the 3s limits (two-sided). Using this approach, it was possible to calculate LOD values of 0.12% for NIR and 0.16% for MIR. This example illustrates that very low LOD can be calculated using spectroscopy-based and regression models. The authors did not use an independent test set for the calculation, and the comparison of the methods on the basis of other parameters such as the RMSE values was not the focus. It is less possible to compare the two studies because the calculations of the LODs or MDLs are based on different mathematical calculations. Moreover, the aim in the present study was to include not only the MDLs but also other parameters such as RMSE values from the individual spectroscopic techniques in the assessment of the quality of the models.

McDowell et al. (2018) described the detection of refined sunflower and rapeseed oil in cold-pressed rapeseed oil by MIR and Raman spectroscopy (McDowell et al. 2018). The loading plots of the PCA of the spectra of both techniques show a similar picture as in the present study. Differences between sunflower and rapeseed oil are particularly evident in the fatty acid distribution, additionally in the areas revealing the specific bands for the pigments. Using MIR spectroscopy, better R^2_{Pred} and the MDL values (R^2_{Pred}: 0.99, MDL: 9% w/w) were calculated compared to analysis by Raman spectroscopy (R^2_{Pred}: 0.96, MDL: 15% w/w), which is in the same range to the present study. In a further study, McDowell et al. (2019) calculated the MDLs for ^{1}H-NMR spectroscopy for the identical authenticity questions and sample sets. For this and in contrast to the present study, three specific regions in the spectrum were used (0.52–3 ppm, 3.9–4.56 ppm, and 4.94–5.8 ppm), resulting in 3217 data points. Normalization to the glycerol signal (3.9–4.56 ppm) was subsequently performed (the same for the present study). For the detection of sunflower oil in rapeseed oil, the MDL of 8% (w/w) and an R^2_{Pred} of 0.99 were determined using PLS-R (McDowell et al. 2019). Accordingly, the lowest MDL and highest RMSE values were also obtained for ^{1}H-NMR analysis.

Alonso-Salces et al. (2022) determined the content of adulterant in olive oil samples, adulterated with various vegetable oils (sunflower oil, hazelnut oil, etc.), by ^{1}H-NMR spectroscopy and PLS-R analysis (Alonso-Salces et al. 2022). The authors carried out the same sample preparation as described in the present study. Normalization to the glycerol signal was also performed. However, Alonso-Salces et al. used a bin width of 0.02 ppm (bucketing) and autoscaling or centering as pre-processing steps. Depending on the adulterant and the degree of adulteration, RMSEP values between 0.32 and 3.4 (% vegetable oil) were determined. Furthermore, detection limits between 2 and 5% (depending on the type of adulteration) were calculated (in the range of the present study), but the evaluation of these limits was not described in the publication.

In summary, the values generated in the present study (RMSEP, RMSECV, MDL) are basically comparable or in a similar range as described in the literature. However, the studies described above illustrate that there are very different approaches for the quantitative determination of adulterants in edible oils. These procedures differ in spectrum evaluation (e.g., selection of specific, spectral regions or bucketing), in model building, optimization and validation, type of model (e.g., PLS-R, OPLS), or in the calculation of detection levels (LOD, MDL). A unified approach does not yet exist, which complicates the comparison of results with other techniques or authenticity questions.

Applicability for Laboratories (as Screening Methods)

With regard to the quantification results and the discussion with existing literature, it is not possible to provide a uniform conclusion concerning which spectroscopy-based method is the most suitable, since this depends strongly on the authenticity question to be investigated. Therefore, this study also considers general as well as environmental aspects, which are shown in Table 2.

A prerequisite for the application of the three spectroscopic techniques is the need for trained personnel who are knowledgeable about the techniques (operation, setting of parameters, etc.), to be able, for instance, to detect any solvent residues (from cleaning procedure).

In order to detect food fraud at an early stage and therefore to be able to act quickly before food products reach

Table 2 Comparison of the three investigated spectroscopic techniques taking into account general aspects as well as environmental aspects, particularly with regard to the analyzed authentication issue

	Spectroscopic method		
	MIR spectroscopy	Raman spectroscopy	^{1}H-NMR spectroscopy
General aspects			
Principle	Molecular vibrations	Light inelastic scattering	Spin transition
Determination of content of specific components	✓	✓	✓
Structure elucidation	✓	✓	✓
For investigated example			
Sample preparation**	✗	✗	✓
Use of hazardous chemicals during sample preparation	✗	✗	✓
Use of hazardous chemicals during measurement	✓	✓	✗
Time of analysis	~ 2 min	~ 2 min	~ 20 min
Automatization of analysis (time saving)	✗	✗	✓

✗: no; ✓: yes; **excluding homogenization

the market, fast and simple analytical applications are necessary. Compared to NMR spectroscopy, MIR and Raman spectroscopy offer significant advantages. Sample preparation does not need to be performed. Moreover, a single MIR and Raman spectroscopic measurement takes approximately 2 min. In addition, there are handheld devices that can be taken on site (during the sampling) to perform the measurements onsite. With an adapted cloud-based solution, the evaluation could be performed in minutes. However, one disadvantage (when a large number of samples need to be measured) is that the instruments are not equipped with an autosampler and therefore each sample must be manually placed on the ATR crystal or in the glass cuvette. In NMR spectroscopy, on the other hand, autosamplers are available, which saves a considerable amount of time in the laboratory. Furthermore, NMR spectral data provide information about individual fatty acids (Guillén and Ruiz 2003a, 2003b), while MIR and Raman data do not. It is also possible to identify and determine from an obtained spectrum compounds (phenols, aldehydes) that play a significant role in sensory properties of an oil or indicate a progressing oxidation of the oil.

Nevertheless, with regard to environmental aspects, the advantages for Raman and MIR outweigh those for NMR spectroscopy. Sample preparation for NMR analysis requires in this particular example deuterated chloroform, which is hepatotoxic and likely carcinogenic. The other two techniques preclude the use of hazardous chemicals during the sample preparation. After a measurement by MIR and Raman spectroscopy, only the ATR crystal and the glass cuvette need to be cleaned with ethanol, which is much less hazardous than deuterated chloroform.

For the specific example, based on the PLS-R results (2 × RMSEP) and considering the described environmental aspects, MIR spectroscopy is recommended for the routine and official control to get a fast first inside about the authenticity of the respective edible oil sample. The samples could be measured directly after receipt in the laboratory without further sample preparation within a short time, evaluated and, if adulteration is suspected, analyzed for clarification using accredited, chromatographic methods according to AOAS (Official Method, 2009/7th). These techniques would help food inspectors detain suspect edible oil samples early, before it is available to consumers.

Conclusions

The results of this study demonstrate that it is possible to detect below 10% refined rapeseed oil as an adulterant in pumpkin seed oil using spectroscopic methods and chemometric evaluation. The lowest contents of adulterant could be detected by ^{1}H-NMR analysis (MDL 3.4% w/w). For MIR and Raman spectroscopy, minimum detection limits of 4.8% w/w and 9.2% w/w were obtained. MIR and Raman spectroscopy are more efficient as screening tools than ^{1}H-NMR analysis, since these two methods do not require sample preparation (only homogenization). This also applies for the commonly employed chromatographic techniques, which demand significantly more laboratory effort and therefore are more time-consuming. To challenge the obtained performance of the models, a larger independent extra test set should be analyzed.

There is a large variety of literature describing the quantification of adulteration in edible oils, but approaches differ in terms of the number of samples included for model calculation and development and basis of calculation for MDL as well as the performance parameters used to assess the mathematical models. Therefore, to harmonize spectroscopy-based methods in combination with multivariate data

analysis, the future focus should be on developing a uniform approach regarding model development, evaluation strategies, and calculation of minimum detection limits with the aim to better compare results generated by different spectroscopic techniques.

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Code Availability Not applicable.

Author Contributions Carolin Lörchner: investigation, formal analysis, writing - original draft; Carsten Fauhl-Hassek: project administration, supervision, writing - review and editing; Marcus A. Glomb: supervision, writing - review and editing; Vincent Baeten: supervision, writing - review and editing; Juan A. Fernández Pierna: formal analysis, writing - review and editing; Susanne Esslinger: conceptualization, writing - review and editing.

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Data Availability Not applicable.

Declarations

Conflict of Interest Carolin Lörchner declares that she has no conflict of interest. Carsten Fauhl-Hassek declares that he has no conflict of interest. Marcus A. Glomb declares that he has no conflict of interest. Vincent Baeten declares that he has no conflict of interest. Juan A. Fernández Pierna declares that he has no conflict of interest. Susanne Esslinger declares that she has no conflict of interest.

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4.2 *Quality control of spectroscopic data in non-targeted analysis*

Publikation B - *Quality control of spectroscopic data in non-targeted analysis – development of a multivariate control chart*

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Quality control of spectroscopic data in non-targeted analysis – Development of a multivariate control chart

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ABSTRACT

In the presented study, an easy to implement workflow based on the evaluation of a quality control sample in non-targeted analysis (outlier detection and time related trend) is proposed for the first time. The novel concept was developed and demonstrated with Fourier transform-midinfrared spectroscopy using a rapeseed oil as quality control sample. Different data evaluation strategies for outlier detection were tested and compared: (i) principal component analysis (PCA), (ii) PCA combined with Hotelling's T-squared distribution and Q-residuals for data assessment as well as (iii) various outlier score-based methods. The build models were challenged by varying measurement and storage conditions to verify the applicability of the three evaluation types (i-iii) to identify these artificially induced variations as outliers. Analogous to a control chart in targeted analysis warning and action limits (numerical decision criteria) were calculated using outlier score-based methods. The best results were achieved by the four outlier score-based methods (pre-period n = 25), where 100 % of the deliberately generated outliers were identified as such.

1. Introduction

Targeted and non-targeted analytical methods are commonly applied to prove the authenticity of a product (Esslinger et al., 2014). Currently, mainly targeted analytical methods are used in official and routine control for food and feed. This is caused by the fact that there are barely legal requirements and standards for non-targeted analysis in contrast to targeted analysis. These requirements have been established to harmonise analytical methods, quality assurance (QA) measures and thereby strengthen the overall validity and reliability of analytical results (Commission, 2002; DIN EN ISO/IEC 17025:2018–03, 2018; DIN ISO 5725, 1994). As part of these regulations, the requirements for internal quality control measures are also specified. One example of this measure is the use of a quality control (QC) sample, which serves as a regular (daily) check of the entire analytical procedure including sample preparation as well as measurement and allows for the rapid identification of time and/or instrumental trends or outliers (Esslinger et al., 2014). The investigations of whether a trend over time or outliers are apparent in the targeted analysis is determined with the application of univariate control charts e.g. according to Shewhart *et al.* (Shewhart & Deming, 1939). In recent years, different control charts have been developed depending on the type of QC sample, e.g. whether a certified or in-house reference material is involved (DIN ISO 7870–1:2019, 2021; DIN ISO 7870–2:2013, 2021).

Although targeted analysis offers a wide variety of advantages like the reliable and specific identification as well as quantification of analytes in numerous matrices, there might remain the disadvantage that only known substances can be investigated. However, this often makes targeted approaches unsuitable to uncover fraudulent and deceptive practices at an early stage. In these cases, changes to the product that are not expected, such as adulteration of products with unknown ingredients, must also be detectable. For this purpose, non-targeted analysis is increasingly moving into the focus of the scientific community in order to detect also unknown substances. Nevertheless, there are limitations in the routine application of these analytical strategies. For example, principles of QA still need to be addressed here (Horn et al., 2019). This includes aspects such as the maintaining of control charts based on QC samples. In contrast to how these issues are handled in

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targeted analysis, non-targeted analysis uses information from the entire spectrum (consisting of several hundred variables) for data evaluation and the majority of analytes are unknown, which requires new approaches to QA (Sangster et al., 2006).

In the field of metabolomics, current studies are focusing on aspects of QA, but it is not possible to directly transfer the published approaches to non-targeted FT-IR analysis (Beger, 2018; Beger et al., 2019; Broadhurst et al., 2018; Dunn et al., 2017; Oberacher et al., 2020). Here, QC samples mainly serve to control the extent of loss to which an analyte experienced during sample preparation. The group of Sangster et al. was the first in the field of metabolomics proposing a non-targeted evaluation of QC measurements using multivariate statistics to identify time trends and outliers (Sangster et al., 2006). This approach has also been used in spectroscopic applications in combination with non-targeted analysis in a few studies. These studies reported the use of QC samples for each measurement-day in non-targeted analysis to regularly check the method performance (Achten et al., 2019; Horn et al., 2018; Nietner et al., 2013). This was performed by calculating a principal component analysis (PCA) as explorative data analysis of QC and commercial samples, followed by a visual inspection (e. g. clustering) of scores plots.

In the field of computer science, similar issues related to the aspect of outlier identification are addressed. Here, the approaches are mainly based, for instance, on the algorithms *k*-nearest neighbour (*k*-NN) (Kordos et al., 2010) or local outlier factor (LOF) (Breunig, Kriegel, Ng, Sander, 2000), so-called outlier score-based methods (Barnett & Lewis, 1984; Breunig, Kriegel, Ng, Sander, 2000). These algorithms are categorised into: i) distance-based (e.g. LOF) (Breunig, Kriegel, Ng, Sander, 2000), ii) density-based (e.g. *k*-NN) (Kordos et al., 2010) and Euclidian distance (ED) (Chandola & Kumar, 2009) and iii) probability-based methods (e.g. kernel density estimation, KDE) (Turlach, 1993). Depending on the applied algorithm, the outlier score calculated for each data point helps in interpreting measurements as inliers or outliers. According to that, an inlier is defined as a measurement that is not an outlier. These outlier score values, describing the distance of a measurement to the centre of a data set. This approach might be adaptable to establish control charts in non-targeted analysis.

Thus, the aim of this study was to propose a standardisable procedure for maintaining a multivariate control chart in agreement to the requirements and regulations in targeted analysis. For this purpose, different mathematical outlier detecting approaches (PCA, PCA in combination with Hotelling's T-squared distribution and Q-residuals and four different outlier score-based algorithms) were investigated using an in-house QC sample and Fourier transform-midinfrared (FT-MIR) spectroscopy.

Through the output of the outlier score-based methods a control chart could be established for the first time in non-targeted analysis.

2. Material and methods

2.1. Sample collection and preparation

The basic prerequisite for performing the approach using PCA according to Sangster *et al.* (Sangster et al., 2006) is to compare the variation of an authentic sample set with measurements of the QC sample. For this purpose, an edible oil was selected as a QC sample that, as far as possible, fulfils the requirements for long term stability (no significant change due to oxidation) and homogeneity. Therefore, a refined rapeseed oil without suspended particles purchased in 2017 from local market (Germany) was used. In addition, the mentioned PCA-based approach requires a set of samples that covers the natural variation within the investigated matrix. For this, an exemplary authentication issue - seed oil variety differentiation by PCA was chosen and respectively, 150 pure seed oil samples (Table S1 in the Supplementary material) were purchased in the German retail trade from 2017 to 2019. The sample set comprised 16 different oilseeds and was intended to represent the botanical diversity of seed oils available in the German retail trade. Within the individual botanical groups, several diversities were considered: different producers, organic and conventional agriculture, the type of pressing, as well as the pre- and post-treatment of the respective raw materials and oils.

The samples were homogenised with an overhead shaker (Reax 2, Heidolph Instruments, Schwabach, Germany) for 30 min at 40 rounds per minute and 21 °C. Aliquots of the sample were filled in 1.2 mL cryogenic tubes (neoLab Migge GmbH, Heidelberg, Germany) and were finally stored under dark conditions at −18 °C excluding any headspace volume in order to prevent oxidation reactions.

2.2. Quality control sample and experimental setup

The QC sample was analysed by FT-MIR spectroscopy every measurement day (at the beginning, the middle and the end of each batch, approx. every 10th sample measurement) under standard conditions (e. g. 30 °C, n = 57, Table 1). All these measurements were acquired within a time period of 36 months and by two different operators ($V_{standard}$: measurements of operator 1 and $V_{operator}$: measurements of operator 2) (Table 1). In addition, the pre-period and seed oil sample set was also measured under these standard conditions.

Some mathematical approaches, tested in this study, are based on the establishment of a pre-period of QC sample measurements to calculate warning and action limits. In order to verify the influence of the respective selection of QC sample measurements, the number of measurements (n = 15, n = 20, n = 25, n = 30) was varied. To challenge the PCA-based and outlier scores-based approaches, measurement parameters were varied by predicting e.g. QC sample measurements, analysed under non-standard conditions (deliberately generated outliers) (Table 1). For example, the abbreviation VT includes the intentional variation of the measurement temperature with $VT_{20°C}$ (8 measurements (m = 3) at a temperature of the diamond crystal of 20 °C), $VT_{25°C}$ (10 measurements (m = 3) at a temperature of 25 °C), $VT_{35°C}$ (10 measurements (m = 3) at a temperature of 35 °C), and $VT_{40°C}$ (10 measurements (m = 3) at a temperature of 40 °C). The edible oil samples chosen for the variation of different seed oil varieties (rapeseed oil ($VV_{rapeseed}$), pumpkin seed oil ($VV_{pumpkin}$) and sunflower oil ($VV_{sunflower}$)) were selected from the described sample set in 2.1.

2.3. FT-MIR spectroscopy

The infrared spectra were recorded on two Nicolet 6700 series spectrometers (Thermo Fisher Scientific, Waltham, USA) in accordance with the procedure described elsewhere (Achten et al., 2019; Nietner et al., 2013).The instruments were equipped with a single attenuated total reflectance (ATR) diamond crystal, a potassium bromide (KBr) beamsplitter and a deuterated triglycine sulfate (DTGS) detector. The optics were continuously flushed with dried nitrogen gas (purity 5.0). Spectra were recorded in triplicate in the absorbance mode from 4000 to 550 cm^{-1} with a spectral resolution of 4 cm^{-1} (data spacing of 1,928 cm^{-1}, Happ-Genzel apodisation) by accumulating 32 scans. All measurements were carried out at (20 °C, 25 °C, 30 °C, 35 °C, 40 °C) ± 0.1 °C maintained with a DC-50-K10 temperature control unit (Thermo Fisher Scientific, Waltham, USA). For each measurement, 1 μL of sample was deposited on the ATR crystal surface. A background spectrum (laboratory air) was recorded and each sample was subsequently measured against its particular background spectrum. After each measurement, the diamond crystal was repeatedly cleaned with a cellulose tissue soaked in *n*-hexane (99.0 %, Merck KGaA, Darmstadt, Germany), methanol (99.9 %, Merck KGaA, Darmstadt, Germany) and acetone (≥99.9 %, Merck KGaA, Darmstadt, Germany) in a three-step procedure and dried. Background spectra were visually checked to exclude solvent and sample residues. The spectrometers were controlled regularly with a polystyrene ATR standard (Thermo Fisher Scientific, Waltham, USA) as reference sample for spectral resolution, signal-to-noise-ratio, and wavenumber accuracy to ensure the stability of the instrument during

Table 1
Variations and number of measurements of QC sample and variations of edible oil sample.

sample name	number of measurement (m = 3)	conditions			
		measurement temperature	operator	storage conditions	seed oil variety
pre-period	15-30	30 °C	1	−18 °C	rapeseed
$V_{standard}$	27–42	30 °C	1	−18 °C	rapeseed
$V_{operator}$	10	30 °C	2	−18 °C	rapeseed
$VT_{20°C}$	8	20 °C	1	−18 °C	rapeseed
$VT_{25°C}$	10	25 °C	1	−18 °C	rapeseed
$VT_{35°C}$	10	35 °C	1	−18 °C	rapeseed
$VT_{40°C}$	10	40 °C	1	−18 °C	rapeseed
VS_{7d}	10	30 °C	1	26.0 C; 7d	rapeseed
VS_{14d}	10	30 °C	1	26.0 C; 14d	rapeseed
VS_{21d}	10	30 °C	1	26.0 C; 21d	rapeseed
VS_{28d}	10	30 °C	1	26.0 C; 28d	rapeseed
$VV_{rapeseed}$	10	30 °C	1	−18 °C	rapeseed
$VV_{pumpkin}$	10	30 °C	1	−18 °C	pumpkin
$VV_{sunflower}$	10	30 °C	1	−18 °C	sunflower
$V_{instrument}$	10	30 °C	1	−18 °C	rapeseed

Variation of $V_{standard}$ = Measurement over a time period of 36 months using standard parameters; $V_{operator}$ = operator using standard parameters; VT = temperature; VS = storage (26.0 °C ± 0.5 °C); $VV_{rapeseed}$ = variety rapeseed oil; $VV_{pumpkin}$ = variety pumpkin seed oil, $VV_{sunflower}$ = variety sunflower oil; $V_{instrument}$ = measurements on second, identically constructed FT-MIR instrument.

the measurements. Data handling was performed with the OMNIC 7.4 software package (FT-MIR 1) and OMNIC 8.3 software package (FT-MIR 2, data set $V_{instrument}$) (Thermo Fisher Scientific, Waltham, USA).

2.4. Multivariate data analysis

Multivariate data analysis was carried out by PLS toolbox version 7.0.3 (Eigenvector Research, Wenatchee, WA, USA) in combination with Matlab version 7.11.0 R2010b (The MathWorks Inc., Natick, MA, USA), KNIME 4.1.1 (KNIME AG, Zürich, Switzerland) and the scikit learn package from Python 3.6.5 (Python Software Foundation).

2.4.1. Preprocessing and data reduction

For all multivariate calculations, the triplicate spectra of each sample were averaged prior to chemometric analysis. In this study, common preprocessing methods for FT-MIR data were chosen based on previous studies (Achten et al., 2019; Horn et al., 2018; Nietner et al., 2013). Due to the low variance in PCA, normalisation (ester band 1800-1700 cm^{-1}), first Savitzky-Golay derivative (Filter Width 15; Polynomial 2) and mean centering were applied on raw spectra as the optimal preprocessing (PP). Wavenumber regions which did not provide relevant spectral information (4000 cm^{-1}-3040 cm^{-1} and 1625 cm^{-1}-1490 cm^{-1} baseline area, 2790 cm^{-1}-1790 cm^{-1} absorption of diamond crystal) were excluded. In total, 701 data points were used for data evaluation for each spectrum.

2.4.2. Explorative data analysis by principal component analysis

Two approaches were performed on principal components, using the singular value decomposition (SVD) algorithm.

1) PCA was calculated according to Sangster *et al.* (Sangster et al., 2006) with the entire set of samples (n = 150) and QC samples (n = 57, pre-period and $V_{standard}$). Resulting score plots were used to evaluate the variation of the sample set and to estimate the model's sensitivity.
2) The pre-period measurements of the QC sample (n = 15, because no improvement of the results was obtained by extending the pre-period, results are not considered further) were used to calculate a second PCA. Considering student's t distribution and the Hotelling's T^2 probability distribution, confidence intervals (CI) were estimated and set as decision criteria for outlier identification, corresponding to the warning and action limits (95.5 % and 99.7 %). In addition, Hotelling's T^2 probability distribution in combination with the Q-statistics (as influence plots) were used (Hotelling, 1992; Joe Qin, 2003) for the detection of potential outliers and compared with the scores plot. To challenge the system, QC measurements (mean spectrum of a triplicate), which were not performed under standard conditions (Table 1), were calculated into this PCA model. The scores and influence plots were used to evaluate the data.

2.4.3. Outlier score-based methods

For the third approach various outlier score-based methods were tested. Based on observed measurement deviations (e. g. standard deviation), warning and action limits were calculated. These limits depend on the outlier score-based method, as these approaches are relying on different algorithms.

2.4.3.1. Distance-based methods – k-nearest neighbour (k-NN) and Euclidian distance (ED). The *k*-NN approach describes a distance-based method where the outlier score can be determined, e. g. by taking the average distance to all *k*-nearest neighbours (Kordos et al., 2010). The ED method is also based on the calculation of distances between two points, in this study the distance between the mean of the pre-period and a sample of variation is calculated (Chandola & Kumar, 2009).

2.4.3.2. Density-based method – local outlier factor (LOF). An advantage/contribution of LOF (and other related methods) is to provide a normalisation of outlier scores for a given data set. In contrast to the outlier score, calculation described in chapter 2.4.3.1 where only absolute distances are used, the LOF also takes the possible different densities in different areas of the 'measurement space' into account (Breunig, Kriegel, Ng, Sander, 2000).

2.4.3.3. Probability-based method – Kernel density estimation (KDE). KDE is based on a probability density function (Turlach, 1993). This method offers the advantage that no specific distribution has to be chosen in advance. Often a normal distribution of the data is assumed and the parameters are set accordingly (Miljkovic, 2010). Similar to *k*-NN and ED, this algorithm also includes the distances to the neighbours in the calculation.

2.4.3.4. Warning and action limits. For the calculation of warning and actions limits, the outlier score values for each outlier scores-based method were calculated based on the pre-period (n = 15, n = 20, n = 25 and n = 30) (Table S2/S3/S4/S5 in the Supplementary material). These outlier score values were averaged (calculated as $\bar{x}$) and standard

deviations were calculated, respectively. Depending on the algorithm, the limits were determined according to $\bar{x}$ of pre-period + 2 or 3 times the standard deviation (for ED, *k*-NN and LOF) or $\bar{x}$ of pre-period – 2 or 3 times the standard deviation (for KDE). To proof the system for applicability, the outlier score value was determined for each QC sample measurement (Table S6/S7/S8/S9 in the Supplementary material) for each outlier score-based method, respectively.

2.4.3.5. Implementation in a Konstanz Information Miner (KNIME)-Workflow. The calculation of the previously described procedures (preprocessing, calculation of the outlier score-based methods) was realised by means of a workflow in KNIME analytics platform. In addition to the visual, documented structure, which is easy to handle even for non-programmers, the workflow (Berthold et al., 2009) might be adapted interactively by other laboratories. Due to the lack of individual calculation bases in KNIME, Python was also used. Please note that the specified version of Python must be applied.

3. Results and discussion

3.1. Aspects of quality assurance

3.1.1. QC sample requirements

To guarantee that a QC sample is suitable for checking the performance of the entire analytical procedure, certain criteria must be met. One criterion is that the QC sample should be as similar as possible to the investigated matrix (Esslinger et al., 2014) in order to adequately represent the properties of the matrix and thus enable quality control of the entire analytical process (from sample preparation to data analysis). In targeted analysis, certified or in-house matrix reference materials with known analyte contents are preferred used as QC samples. However, they are only available for a limited number of analyte-matrix combinations. Regarding non-targeted approaches, such materials are not yet commercially available. Nevertheless, there are currently in-house QC sample strategies, which have successfully been applied in literature. Sangster *et al.* (Sangster et al., 2006) used a QC-pool sample, consisting of mixed aliquots from every sample of a given and finalised sample set, which is a common procedure in metabolomics. This type of QC sample aims to combine different diversities (individual specificities) of matrix in one sample and thus represents the composition of the entire sample set on the average. For the investigation presented here, the use of a pool sample was discarded: according to the design of the experimental setup, the sample set was continuously expanded over a period of three years in order to cover the greatest possible variation of edible oils (e.g. lot number). Under these circumstances, it is common practice to use a commercial sample as QC sample, which is available in large quantities (Achten et al., 2019; Nietner et al., 2013). To realise this, a single bulk of refined rapeseed oil was selected as the QC sample previously to the analysis of market samples.

A second criterion that must be met is a sufficient short- and long-term stability of the QC sample. Particularly in the case of edible oils, oxidation processes can induce changes in the chemical composition and thus result in changes in the spectra. In order to demonstrate that the chosen QC sample is stable enough, Fig. 1 illustrates all measurements of the QC sample in a line plot. The spectra of the QC sample show significant bands at 2924 cm^{-1} and 2854 cm^{-1} (1/2: asymmetric and symmetric stretching vibration of the aliphatic CH_2 functional group) as well as at 1746 cm^{-1} (3: ester carbonyl functional group of the triglycerides) (Guillén & Cabo, 1999; Guillén & Cabo, 2000; Vlachos & Arvanitoyannis, 2008).

Spectral changes stemming from oxidation processes would be accompanied by a shift in frequency of the maximum absorbance, an increase (e. g. at 1745 cm^{-1}) or decrease (e. g. at 3006 cm^{-1}) in absorption or widening of specific, spectral bands (e. g. at 1745 cm^{-1}), depending on the degree of oxidation (Guillén & Cabo, 1999; Guillén & Cabo, 2000; Muik, B., Lendl, B., Molina-Díaz, A., Ayora-Cañada, M.J., 2021; Vlachos & Arvanitoyannis, 2008). These described changes were not observed in the superimposed spectra of all measurements during the three-year measurement period and thus, the QC sample was confirmed to be stable over time.

3.1.2. QC sample measurement requirements

A decrease in intensity over the entire spectrum was identified within the three-year measurement period (Fig. 1). Measurements of the QC sample in 2017 (grey lines) show consistently higher intensities over the whole spectrum than measurements performed in 2018 (green lines) and 2019 (black lines). Since the intensities decrease over the whole spectrum and were not restricted to specific bands, an oxidation process of the sample can be excluded. Investigations and comparisons with a reference sample (polystyrene ATR standard) and a new IR source have shown that this effect is due to the ageing of the source. This effect could be explained by the increasing age of the source as described by Feudale *et al.* (Feudale et al., 2002).

However, for the QA approaches tested in this study, the intensity

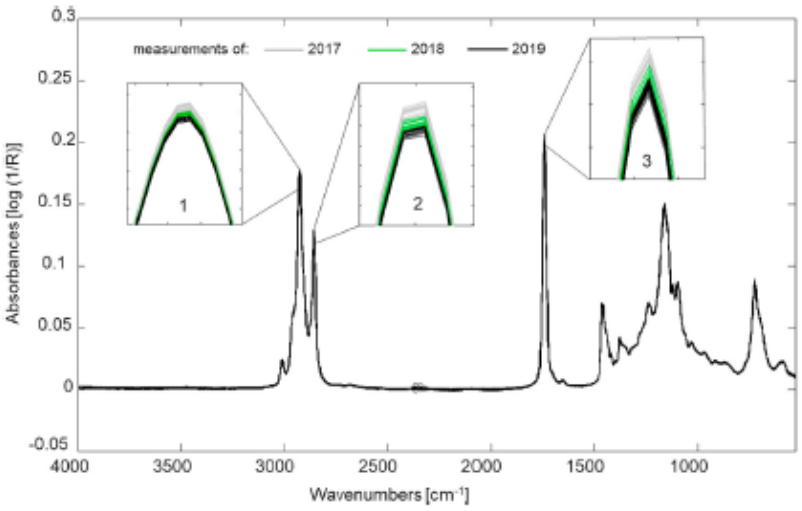

Fig. 1. Line plot of QC sample measurements (n = 57, m = 3) over a period of 36 months, pre-period 2017 (n = 15; grey line), QC 2018 (n = 25; green line), QC 2019 (n = 17; black line) – region 1/2: at 2925 cm^{-1} and 2854 cm^{-1} (symmetric and asymmetric stretching vibration of the aliphatic CH_2 group); region 3: at 1746 cm^{-1} (ester carbonyl functional group of the triglycerides) (Guillén & Cabo, 1999; Guillén & Cabo, 2000). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

decrease over time might be problematic. Samples measured at a later stage might mistakenly be declared as outliers due to the intensity decrease caused by ageing of the IR source. This effect was therefore compensated by normalisation of spectral data. For this approach, there are two possible options: either normalisation to the total intensity (entire spectrum) or normalisation to a selected band. In contrast to normalisation to the entire spectrum, normalisation to the 1725 cm^{-1} band allows the detection of an incipient oxidation process (Guillén & Cabo, 1999; Guillén & Cabo, 2000; Guillén & Cabo, 2002). Accordingly, latter approach was chosen. The ratio of the intensities in the whole spectrum were changed and intensity differences at 1725 cm^{-1} were eliminated.

3.2. Explorative data analysis - PCA

In order to carry out initial quality-assured measures in non-targeted analysis, explorative data analysis (e.g. PCA) is applied to monitor potential time or analytical drifts, observe systematic instrument changes and thus to investigate the variation of measurement outliers during one or between several sequences (Horn et al., 2018; Nietner et al., 2013). The PCA scores plot of the first two PCs (Fig. 2a), representing 92 % of the total variance, shows the overall variation of the FT-MIR spectroscopic data of the entire set of commercial edible oil samples (n = 150) and the QC samples (n = 57) obtained over the course of 36 months (Chapter 2.2). The replicates of the QC sample (white squares) vary much less in the PCA scores plot than the measurements of commercial edible oil samples (grey diamonds). The described type of evaluation was initially published by Sangster *et al.* (Sangster et al., 2006). The authors postulated, that an analytical method can be applied for a specific problem, e.g. differentiation of variety of edible seed oil, if the analytical variation represented by the QC samples is visually smaller compared to the product variation of the commercial samples in the PCA scores plot. According to that, the data presented in this study fulfil this requirement and thus can be used for further evaluation. To challenge this model, deliberately generated outliers (under different measurement conditions, here 20 °C instead of 30 °C) were calculated into the PCA. The PCA scores plot (PC1 and PC2) is shown in Fig. 2b. The QC samples measured at a temperature of 20 °C (black triangles) are grouped along PC1 to the right of the group of QC sample replicates (white squares) measured under standard conditions. Nevertheless, the variation of the replicates of both variations of the QC sample (white squares and black triangles) is smaller than the measurements of commercial edible oil samples (grey diamonds) and thus still fulfil the requirements according to Sangster *et al.*.

However, also for the evaluation according to Sangster et al., a representative sample set covering the natural variation of the matrix under investigation must be available (within the defined scope according to the research question) (Donarski et al., 2019) before the analysis and assessment of variation of the QC samples can be performed. Since QA measures have to be implemented from the beginning of the study, this approach is not practicable.

An even greater disadvantage of data evaluation by means of PCA is that this approach is based only on a purely visual evaluation and consequently on the expert knowledge of the analytical chemist. There is a strong need for a numerical evaluation in which limit values are also defined as criteria for outlier detection.

3.3. Explorative data analysis - PCA in combination with Hotelling's T^2- and Q-statistics

For this PCA-based approach in combination with Hotelling's T^2-and Q-statistics, the calculation was based on a defined pre-period (2.4.2) of the QC sample measurements (n = 15, m = 3). Information from Hotelling's T^2 and Q-residuals were used within a respective influence plot to identify outliers in the data set at the 95.5 % and 99.7 % significance level (calculated using Hotelling's T^2) as warning and action limits, respectively. Furthermore, the influence plots were used to obtain the difference or residual, which is not described by PCA but by means of the Q-residuals.

Afterwards to the calculation of the warning and control limits, "new" measurements of the QC sample were included separately into the PCA model to challenge the system. Fig. 3 shows the PCA scores plots obtained by the first two PCs (a and c), which represent 74 % of the total variance and the influence plots (b and d) with defined pre-period (n = 15) incl. one example of QC measurement variation (VT3, at 35 °C). The measurement (black square) lies outside the dashed grey line in Fig. 3a and b and thus outside of the 95.5 % CI, but within 99.7 % CI, in Fig. 3c and d. In general, the measurements are categorised according to the following decision criteria. A measurement is identified as an inlier that is inside the CI at 95.5 % and thus, no corrective action is required. If a measurement is between both limits (as shown in Fig. 3), the measurement is recognised as a suspect measurement, which provides an indication of a potential analytical problem. A measurement is identified as an outlier that lies outside the CI at 99.7 %. The respective spectrum shows "deviations from the reference" and corrective action, such as control and (if necessary) adjusting of the measurement temperature, must be taken to avoid further outliers. Thus, subsequent measurements are under particular observation. Regarding the present study, it could be stated that the measurement of $VT_{35°C}$ is not an outlier from a purely mathematical point of view (based on CI 99.7 %) and respective data can

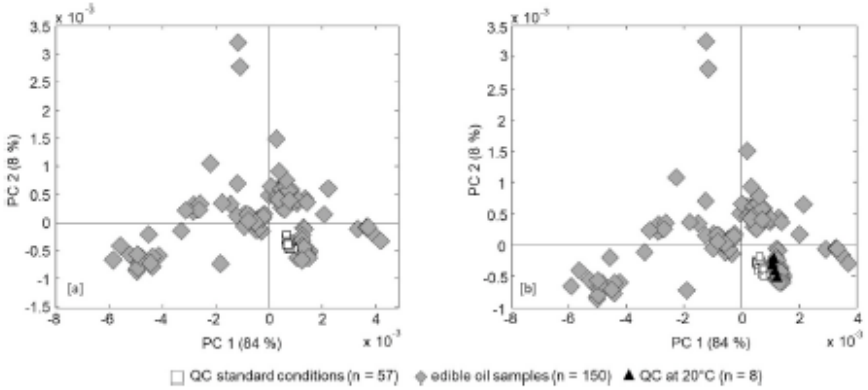

Fig. 2. Scores plot (PC1/PC2) of a sample set (n = 150, grey diamonds) with QC sample measurements (n = 57, white squares) after preprocessing; variance of QC samples is lower than the variance of commercial samples; [a] symbolised by type of sample [b] additional with measurements of QC sample (n = 8), measured at 20 °C ($VT_{20°C}$, black triangles).

preferred or at least combined with the information of the scores plot. Nevertheless, the results obtained using the scores and the influence plots showed that these approaches are not sufficiently sensitive at a CI of 99.7 % regarding the application as quality control tool. Moreover, even measurements that lie within the CI can still be outliers (Bro & Smilde, 2014). This approach is only capable to a limited extent for outlier detection and for maintaining a control chart but is an applicable tool to visualise the variation within the data set.

3.4. Evaluation by outlier score-based methods

The group around Shewhart and Deming was one of the first who developed a univariate control chart based on the mean value of QC measurements in process control (Shewhart & Deming, 1939). Shewhart and Deming identified upper and lower warning ($\bar{x}$ of pre-period ± 2 times the standard deviation) as well as action limits ($\bar{x}$ of pre-period ± 3 times the standard deviation). As indications for systematic deviations these limits serve to monitor the daily variation of a single variable in an analytical process. This procedure was also applied and is still current practice in the field of analytical chemistry. This approach was adapted for the present study.

After calculating the warning and action limits (Table S10 in the Supplementary material) based on n = 15 (pre-period), it was observed that the models are very sensitive regarding outlier detection. Measurements performed under standard conditions ($V_{standard}$ and $V_{operator}$) were identified as outliers (above the action limit) with 62 % for LOF, 26 % for ED, 24 % for *k*-NN and 19 % for KDE (Table S13 in the Supplementary material), although no abnormalities in the measurements could be identified, either by visual inspection of the spectra nor by PCA. Therefore, it is particularly important that a sufficiently large number of measurements are included in the pre-period. In order to cover this representatively with a realistic variation, in analogy to targeted analysis (DIN ISO 7870–1:2019, 2021; DIN ISO 7870–2:2013, 2021). The pre-period was extended to n = 20, 25, 30 measurements (Table S11/S12/S14/S15 in the Supplementary material). The best results were obtained with pre-periods from n ≥ 25 measurements. Thus, it is recommended to include at least 25 measurements in the pre-period. In the following, the results with this number of measurements will be presented and discussed. Based on the mean values and respectively determined action and warning limits (Table 3), the outlier scores were calculated for each measurement of QC samples (Table S8 in the Supplementary material).

The higher (for *k*-NN, ED and LOF) or lower (for KDE) an outlier score, the more a measurement deviates from those of the pre-period (Barnett & Lewis, 1984; Breunig; Kriegel, Ng, Sander, 2000; Chandola & Kumar, 2009; Kordos et al., 2010). The outlier scores were subsequently classified according to whether the outlier score was below (for *k*-NN, ED and LOF) or above (for KDE) the warning limit (inlier), between the warning and action limit (suspect measurements) as well as above (for *k*-NN, ED and LOF) or below (for KDE) the action limit (outlier, Table 4).

The evaluations based on the four algorithms provided comparable results. 100% of the measurements performed under standard conditions ($V_{standard}$) were identified as inliers using the four algorithms. These measurements were identified as inliers in the PCA-based approaches as well. Moreover, all measurements carried out by another operator ($V_{operator}$) were calculated as inliers. In addition, 100 % of $VT_{20\,°C}$, $VT_{35\,°C}$, $VT_{40\,°C}$, $VV_{pumpkin}$, $VV_{sunflower}$ and $V_{instrument}$, were identified as outliers. In contrast to that, the PCA-based evaluation identified these measurements as suspect (outside the warning limit, but inside the action limit). This indicates that the evaluation using the outlier score-based methods is more sensitive than the PCA-based evaluation with regard to outlier detection. A possible presentation of a control chart is shown in Fig. 5 based on the ED algorithm as an example.

Table 3
Mean values, standard deviations and warning and action limits based on outlier scores of pre-period QC sample measurements (n = 25, m = 3) for each algorithm.

	ED	*k*-NN	LOF	KDE
Mean	1.820	2.457	0.999	0.078
SD	0.310	0.201	0.015	0.013
Warning limit	2.440	2.860	1.030	0.053
Action limit	2.750	3.062	1.045	0.040

SD = standard deviation; ED = Euclidian distance; *k*-NN = *k*-nearest neighbour; LOF = local outlier factor; KDE = kernel density estimation.

The points of the measurements vary around the mean value of the pre-period. An indication of a trend towards the warning and action limit is not obvious. Comparable plots were obtained for the other three algorithms (Fig. S1/S2/S3 in Supplementary material).

To give an example of an indication of a time trend, the outlier score values of the respective measurements of the storage sample obtained by ED were presented in a mean value control chart (Fig. 6). The value of the outlier score is a measure of the distance between the respective measurement and the centre of the model (calculated mean of outlier scores from pre-period). Nevertheless, it is not possible to use the algorithms to identify the direction of distance in the multidimensional space. This makes it difficult to identify time related trends. Continuously increasing outlier scores merely indicate a time trend. As an example, outlier score values of the measurements of the storage sample were presented in a mean value control chart (based on ED, Fig. 6).

The storage of the QC sample at a temperature of 26 °C for 14 days (VS_{7d}/VS_{14d}) has no apparent effect on the chemical composition of the sample. The respectively calculated outlier scores (VS_{7d}/VS_{14d}) show no trend towards the warning or action limit. From measurement 20 onwards (VS_{21d}/VS_{28d}), the values are rising and thus moving closer to the limits. Furthermore, after determining the mean value of the outlier scores for each storage period, it was observed that this increases constantly with increasing storage time ($VT_{20\,°C}$: 1.781, $VT_{25\,°C}$: 1.942, $VT_{35\,°C}$: 2.231, $VT_{40\,°C}$: 2.307). This increasing distances between these measurements and the pre-period may indicate a respective time related trend. Regarding the spectra, a marginal change in the intensity ratios was observed (e. g. at 3006 cm^{-1}, Chapter 3.1.2), which could indicate oxidation processes during storage (Guillén & Cabo, 1999; Guillén & Cabo, 2000; Muik, B., Lendl, B., Molina-Diaz, A., Ayora-Canada, M.J., 2021; Vlachos & Arvanitoyannis, 2008).

Thus, it can be concluded, that the four algorithms provide comparable results and appear to be appropriate for establishing a multivariate control chart to identify outliers and the indication of a time trend. However, it is recommend using the *k*-NN and ED algorithms for two reasons. First, these two outlier score-based methods are independent of parameters (for *k*-NN: if *k* is defined as the number of measurements in the pre-period) that have to be checked before calculating the algorithm, as is the case with KDE (Miljkovic, 2010). Secondly, they are easier to understand even for non-mathematicians, in contrast to the LOF algorithm, which takes the density of the points into account (Breunig, Kriegel, Ng, Sander, 2000).

4. Conclusion

For the first time, an evaluation strategy for maintaining a control chart in non-targeted analysis was developed on the example of edible oil measurements using FT-MIR spectral data, based on KNIME analytical platform. To build a multivariate control chart for spectroscopic data, PCA-based and outlier score-based methods, were compared. The outlier scores-based methods *k*-NN, LOF, KDE and ED showed that typical measurement variations (e.g. measurement temperature) for FT-MIR could be identified as outliers by applying previously calculated warning and action limits, based on a defined pre-period of measurements. It is recommended to use the two algorithms *k*-NN and ED.

Table 4
Results of outlier detection for the variations of QC sample, considered separately for CI 95.5 % and CI 99.7 %; calculations based on a defined pre-period (n = 25, m = 3) of QC sample measurements.

generated as	sample name	below CI 95.5 % inlier [%]				CI 95.5 % warning limit* suspect measurements [%]				above CI 99.7 % action limit outlier [%]			
		ED	*k*-NN	LOF	KDE	ED	*k*-NN	LOF	KDE	ED	*k*-NN	LOF	KDE
inlier	$V_{standard}$	100	100	100	100	0	0	0	0	0	0	0	0
	$V_{operator}$	100	100	100	100	0	0	0	0	0	0	0	0
outlier	$VT_{20°C}$	0	0	0	0	0	0	0	0	100	100	100	100
	$VT_{25°C}$	0	11	22	11	22	22	0	22	78	78	78	67
	$VT_{25°C}$	0	0	0	0	0	0	0	10	100	100	100	90
	$VT_{40°C}$	0	0	0	0	0	0	0	0	100	100	100	100
	VS_{7d}	100	100	100	100	0	0	0	0	0	0	0	0
	VS_{14d}	100	100	100	100	0	0	0	0	0	0	0	0
	VS_{21d}	100	90	100	100	0	10	0	0	0	0	0	0
	VS_{28d}	90	50	90	90	10	40	0	10	0	10	10	0
	$VV_{rape-seed}$	0	0	0	0	0	0	0	0	100	100	100	100
	$VV_{pump-kin}$	0	0	0	0	10	0	0	10	90	100	100	90
	$VV_{sun-flower}$	0	0	0	0	0	0	0	0	100	100	100	100
	$V_{instru-ment}$	0	0	0	0	0	0	0	0	100	100	100	100

Variation of $V_{standard}$ = Measurement over a time period of 36 months using standard parameters; $V_{operator}$ = operator using standard parameters; VT = temperature; VS = storage (26.0 °C ± 0.5 °C); $VV_{rapeseed}$ = variety rapeseed oil; $VV_{pumpkin}$ = variety pumpkin seed oil, $VV_{sunflower}$ = variety sunflower oil; $V_{instrument}$ = measurements on second, identically constructed FT-MIR instrument; CI = confidence interval; ED = Euclidian distance; *k*-NN = *k*-nearest neighbour; LOF = local outlier factor; KDE = kernel density estimation; * measurements between CI at 95.5 % and CI 99.7 %.

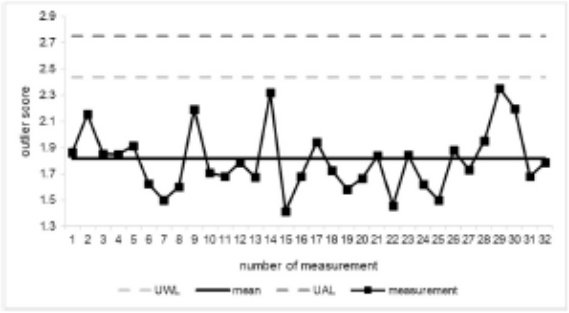

Fig. 5. Mean value control chart based on Euclidian distance method and pre-period (n = 25, m = 3); dashed light grey line - upper warning limit (UWL); dashed dark grey line - upper action limit (UAL); black horizontal line - mean of calculated outlier scores for pre-period measurements; black line with black squares-measurements of $V_{standard}$ (n = 32).

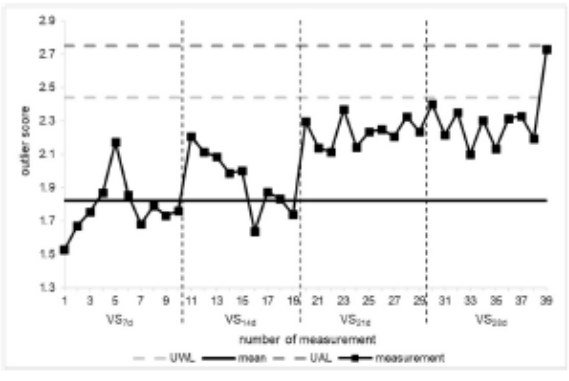

Fig. 6. Mean value control chart based on Euclidian distance method and pre-period (n = 25, m = 3); light grey dashed line - upper warning limit (UWL); dark grey dashed line - upper action limit (UAL); black horizontal line - mean of calculated outlier scores for pre-period measurements; black line with black squares - measurements of VS_{7d}, VS_{14d}, VS_{21d} and VS_{28d} (n = 39).

Furthermore, it is possible, to obtain an indication of a time trend and to monitor the performance of the method based on concrete numerical values. In contrast, PCA-based methods combined with Hotelling's T^2- and Q-statistics were less sensitive regarding outlier detection based on limits as decision criteria. In analogy to control charts in targeted analysis, it can be stated, that the number of QC sample measurements, which are taken into consideration for calculating the pre-period, is crucial for further calculations. This aspect is directly reflected in the robustness/sensitivity of the control chart. In this study, a number of measurements of QC sample is advised at least 25. The presented strategy for quality control is generally applicable and can therefore be adapted to other food matrices and techniques (e.g. Nuclear Magnetic Resonance (NMR) or Raman spectroscopy) as the analytical variation of a pre-period of the QC sample measurements is included in the calculation.

Author contributions

Carolin Lörchner: Investigation, Formal analysis, Writing - Original Draft, Martin Horn: Formal analysis, Writing - Review & Editing, Felix Berger: Investigation, Formal analysis, Carsten Fauhl-Hassek: Project administration, Supervision, Writing - Review & Editing, Marcus A. Glomb: Supervision, Writing - Review & Editing, Susanne Esslinger: Conceptualization, Writing - Review & Editing.

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.

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4.3 *Towards comparable spectra in non-targeted food authentication*

Publikation C - *Towards common useable spectra in non-targeted analysis - a feasibility study by mid-infrared spectroscopy, transfer and correction approaches*

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Towards common useable spectra in non-targeted analysis - A feasibility study by mid-infrared spectroscopy, transfer and correction approaches

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ABSTRACT

Spectroscopy-based methods are often used to verify the authenticity of food and feed. In the scientific environment, especially non-targeted approaches are becoming increasingly popular here, although their routine applicability, e.g., in official food control, is currently limited. The comparability of spectra acquired by different spectroscopic instruments using the same measurement principle is essential for the application of a common spectral database in laboratories and represents a current challenge in this field. In order to investigate possible approaches towards improved comparability of these spectra, a sample set of rapeseed (*Brassica napus*) and pumpkin seed (*Cucurbita maxima*) oils was analyzed using three Fourier-transform-mid-infrared spectrometers following the same procedure for sample preparation and measuring conditions. Depending on the instrument, the obtained spectra exhibited differences in absorbance, but not in wavelengths of bands position and maxima. As a result, the mutual prediction of original data (without mathematical correction) from different instruments using a partial least squares discriminant analysis model built and optimised with spectral data from only one instrument provided a sensitivity of 0% for the two-class model of rapeseed in pumpkin seed oil, indicating no discrimination at all. In order to minimize the spectral differences and thus to enhance the performance parameters of the prediction models, different mathematical correction approaches were investigated: (i) instrument-specific correction factors, (ii) combinations of different pre-processing steps and (iii) piecewise direct standardization (partial least squares-based regression). All investigated approaches achieved classification results with 100% sensitivity for the mathematically corrected data set of the respective instrument. The outcome of this study indicates that the correction approaches can be used to optimize the comparability of spectral data from different instruments, which is a first step towards harmonization in non-targeted analysis.

1. Introduction

The identification of instances of non-compliance with European Union (EU) food legislation, including fraudulent practices, requires effective and reliable routine procedures for official controls [1]. The establishment of these routine applications as well as their uptake are interconnected with appropriate analytical standards which address the current challenges and provide robust, reliable and comparable results [2, 1]. The analytical methods used and the consistency of the resulting analytical data need to be regularly verified and continuously improved to ensure the quality, safety and authenticity of products along global supply chains [1].

In recent years, scientists have recognized that non-targeted analysis has great potential for detecting fraudulent practices regarding the authenticity of products [3,4]. This analytical strategy is based on the acquisition of a so-called chemical fingerprint of the specific food by e.g. spectroscopic methods, such as mid-infrared (MIR), near-infrared (NIR) or nuclear magnetic resonance (NMR) spectroscopy in combination with multivariate data evaluation [5,6]. The fingerprint consists of the entire spectrum and is thus characteristic for a defined matrix. Numerous publications describe fingerprinting analysis based on MIR spectroscopic measurements in the field of authenticity of edible oils. These

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studies present a wide range in terms of application potential (from the evaluation of spectra with multivariate classification to regression models), depending on the authenticity question [7–11]. Nevertheless, all these concepts are (predominantly) isolated solutions, meaning that each institution/laboratory uses a specifically compiled database for comparing the sample with reference data. In the field of NMR spectroscopy, there are established non-targeted, inter-laboratory databases, which contain thousands of wine, honey and juice spectra and provide classification models that can be used for verifying the authenticity of these food matrices (detection of variety, geographical origin or adulteration) [12–14]. A number of other databases are described in the literature [15–18], but these have a different objective and are not based on the application of non-targeted analysis. However, these databases are commercially driven and not fully transparent, which is an impediment to their routine use in official control. For a common useable database, a number of aspects need to be considered [19,20]: authentic and representative samples, covering natural product variation and providing a valid reference for spectra comparison are needed and comprehensive validation strategies (including statistical data evaluation) are required [19,21]. However, one of the greatest challenges in the application of a common database is the comparability of the generated spectral data, which enables the accurate authentication of a sample using databases and valid multivariate models created by another laboratory [22].

In order to minimize spectral differences between data acquired with different instruments (using the same measuring procedure), and thus to be able to use models built and optimized in a different laboratory, various correction approaches have already been discussed in the literature [23–26].

One approach to improve comparability of spectral data was reported for quantitative NMR spectroscopy [27]. This is based on correlating the intensities of a few signals of a specific sample between two spectrometers, and thus calculating a correction factor called Electronic REference To access In vivo Concentrations (ERETIC). In addition to the intensities, other NMR-specific parameters are also included in these calculations. Another study aiming to achieve comparability of spectral data uses the entire spectrum to calculate a factor [28]. The authors examined wine samples measured by NMR spectroscopy and evaluated by means of a classification model.

Another approach to minimize spectral differences was described using optimized pre-processing [29]. Here, baseline correction and area normalization of spectral data, measured on two different MIR spectrometers (with differences of design) were used, in combination with discriminant analysis (DA). In particular, the authors investigated how the number of principal components (PCs) calculated for the model affected the classification rates for the discrimination of raspberry and strawberry purees. Depending on the number of PCs in the DA model, different classification rates were determined. However, according to the descriptions of Holland et al. this approach had a limited applicability, since the pre-processing of the spectra was optimized specifically for this authenticity problem.

Nonetheless, the main focus of the studies, dealing with spectral data comparability/standardization, is on the use of multivariate calibration transfer approaches, which enables the data set of one instrument (called primary instrument) to be transferred to a second instrument (called secondary instrument) by correcting the instrumental differences [30]. Especially in the field of NIR spectroscopy, these methods have been described and compared in reviews [23–26,31–35]. Common calibration transfer methods include orthogonal signal correction (OSC), orthogonal projection, finite impulse response (FIR) or piecewise direct standardization (PDS).

Calibration transfer approaches have so far mainly addressed specific, targeted issues: the quantitative determination of melamine in milk has been investigated and combined with partial least squares regression (PLS) to illustrate the improvement of the model results [36–40]. The European Milk Recording (EMR) network (including a total of 102 MIR instruments) [41]makes particular use of the PDS approach to standardize milk MIR spectra from different laboratories. Using this approach, each wavenumber range of the primary device is associated with a wavenumber window of the respective secondary device. Subsequently, a linear regression is calculated between the spectral response of the primary device of the respective window (spectral range) and the corresponding window of the secondary device. This results in regression vectors (also called standardization coefficients) that vary depending on the primary-secondary combination. Newly measured samples on the secondary instrument are adjusted to the primary instrument using these coefficients. However, the selection of standardization samples (a certain number and type of samples, measured on all instruments and used for the calculation of the coefficients) for PDS calculation is a very crucial step, as these samples must cover the variability of a previously defined matrix to avoid miscalculations.

There is a broad variety of options available for minimizing spectral differences in spectroscopy. The aim of this study is therefore, to compare correction approaches for non-targeted analysis for ensuring the comparability of MIR spectra. This is illustrated by the example of pumpkin seed oil and rapeseed oil samples acquired using different instruments (identical instruments from the same manufacturer and MIR spectrometers from different manufacturers) for their use in a common database. The distinction of pumpkin seed oil and rapeseed oil is selected because the oil spectra of the botanical seeds reveal, for example, clear differences in the range of 1050 cm^{-1}-880 cm^{-1} (bending vibrations of conjugated CH *trans*, *trans*- and *cis*, *trans*-olefinic groups) [42–45]; given the large differences in the fatty acid spectrum, it was therefore assumed that 100% classification is feasible between the two varieties. Three different correction approaches are examined: i) instrument-specific correction factor, ii) data pre-processing (normalization, derivatives) and iii) PDS [46–48]. Based on the results of Principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) [49], the effects of correction approaches are compared and advantages as well as disadvantages are discussed. The authors are not aware of any reference investigating and discussing the optimization of the comparability of spectra generated by different MIR instruments using three different mathematical approaches (uni- and multivariate).

2. Material and methods

2.1. Sample collection and preparation

In 2017 and 2018, 40 rapeseed and 44 pumpkin seed oil samples as well as three sunflower oil and linseed oil samples (Table S1 in the Supplementary material) were purchased from the German retail trade. Within both botanical groups, diversity was sought in the sample set as much as possible by choosing e.g. different producers, organic and conventional cultivation, different types of pressing, and different types of pre- and post-treatment of the respective raw materials and oils.

The purchased samples (in glass or plastic flasks) were homogenized using an overhead shaker (Reax 2, Heidolph Instruments, Schwabach, Germany) for 30 min at 40 rounds per minute at 21 °C prior to analysis. Aliquots of the samples were used to fill 1.2 mL cryotubes (neoLab Migge GmbH, Heidelberg, Germany), taking care to avoid headspace volume to prevent the oil from oxidation. These aliquots were stored in the dark at −18 °C. In order to prepare them for analysis, the samples were tempered to 21 °C.

2.2. MIR spectroscopy

The mid-infrared spectra were recorded on three different instruments (two instruments from the same manufacturer and one MIR spectrometer from another manufacturer).

MIR 1 and 2 (both Nicolet 6700 series spectrometers (Thermo Fisher

Scientific, Waltham, USA)) were equipped with a single attenuated total reflectance (ATR) diamond crystal, a potassium bromide (KBr) beamsplitter, and a deuterated triglycine sulfate (DTGS) detector. The optics were continuously flushed with dried nitrogen gas (purity 5.0). For each measurement, 1 μL of sample was deposited on the ATR crystal surface. Spectra for each sample were recorded in triplicate in the absorbance mode from 4000 to 550 cm^{-1} with a spectral resolution of 4 cm^{-1} (data spacing of 1.928 cm^{-1}, Happ-Genzel apodization), by accumulating 32 scans. All analyses were carried out at 30.0 ± 0.1 °C, maintained using a DC-50-K10 temperature control unit (Thermo Fisher Scientific, Waltham, USA). In accordance with previously published procedures [50, 51], the spectrometer performance was controlled regularly with a thick polystyrene ATR standard (Thermo Fisher Scientific, Waltham, USA) for its spectral resolution, signal-to-noise-ratio, and wavenumber accuracy to ensure the stability of the instrument during the measurements. Data handling was performed with the OMNIC 7.4 software package (MIR 1) and OMNIC 8.3 software package (MIR 2) (Thermo Fisher Scientific, Waltham, USA).

MIR 3 (Vertex 70 spectrometer (Bruker Corporation, Ettlingen, Germany)) was equipped with the standard air-cooled source, a single ATR diamond crystal, a wideband IR beamsplitter, and a room temperature deuterated lanthanum α-alanine-doped triglycine sulfate (DLaTGS) detector. For each measurement, 1 μL of sample was transferred on the crystal surface. Spectra for each sample were recorded in triplicate in the absorbance mode from 3996 to 600 cm^{-1} with a spectral resolution of 4 cm^{-1} (data spacing of 1.928 cm^{-1}, Happ-Genzel apodization) by accumulating 32 scans. Data handling was performed with the OPUS 6.5 software package (Bruker, Waltham, USA).

For all three instruments, a background spectrum (laboratory air) was recorded, and each sample was subsequently measured against its particular background spectrum. After each measurement, the diamond crystal was thoroughly cleaned with a cellulose tissue soaked in *n*-hexane (99.0%, Merck KGaA, Darmstadt, Germany), methanol (99.9%, Merck KGaA, Darmstadt, Germany) and acetone (≥99.9%, Merck KGaA, Darmstadt, Germany) in a three-step procedure and then dried. Background spectra were visually checked for solvent and sample residues.

2.2.1. Quality assurance

For monitoring the performance of the spectrometers, one additional, clear edible oil sample (refined rapeseed oil) purchased in 2017 from the German market was selected as a quality control sample (QC sample). The QC sample was prepared in the same way as the seed oil samples (see 2.1) and was analyzed by MIR spectroscopy on every measurement day at the beginning, in the middle and at the end of each batch and then investigated individually by superimposing all QC spectra, as well as together with the seed oil samples using PCA. The preprocessing of the spectra was performed in advance using normalization (ester band 1725 cm^{-1}), 1st Savitzky-Golay derivative (Filter Width 15; Polynomial 2) and mean centering. Based on the PCA evaluation, any time- or analytically dependent drifts from inter-day variation as well as intra-day variation could be identified.

2.3. Statistical data analysis

Multivariate data analysis was carried out with PLS toolbox version 7.0.3 (Eigenvector Research, Wenatchee, WA, USA) and with Matlab version 7.11.0 R2010b (The MathWorks Inc., Natick, MA, USA). Univariate data analysis was performed using Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA, USA).

2.3.1. Pre-processing and data reduction

Regardless of the correction approach used, the triplicate spectra of each sample were averaged and the resulting mean spectrum was used for all chemometric analyses. As the spectral ranges of MIR 3 were different from those of the other two instruments, the spectral area of 600 cm^{-1} - 550 cm^{-1} was eliminated. Furthermore, wavenumber regions that did not provide relevant spectral information (baseline area at 4000 cm^{-1} - 3045 cm^{-1} and 1625 cm^{-1} - 1490 cm^{-1}, absorbance of diamond crystal at 2790 cm^{-1} - 1790 cm^{-1}) were excluded. Finally, 678 data points for each spectrum were used for data analysis. The spectra (including the original spectra) were also mean centred (column-based centering) before data analysis, which is a common procedure for MIR spectral data [50–52]. In addition, this pre-processing step ensures that the results are interpretable with respect to variation around the mean. This procedure was applied to each dataset (MIR 1, MIR 2 and MIR 3, respectively) including the associated QC samples (for quality assurance). In addition, mean centering was also performed for the entire dataset (MIR 1 + MIR 2 + MIR 3) without QC samples.

2.3.2. Correction approaches

Three correction approaches were investigated: i) instrument-specific correction factors; ii) combinations of different pre-processing steps and iii) calibration transfer using PDS. For the assessment of the results (before and after correction of the respective data set) based on the three correction approaches, the PCA (as a visual tool) and the performance parameters of the PLS-DA model were considered. In what follows, calculations are based on the measurements of MIR 1, which was defined as the primary (reference) instrument. This means that the data set of MIR 1 was used for PLS-DA model building and internal validation. The measurements of MIR 2 and 3 were defined as secondary instruments and adapted to the primary instrument. To investigate the influence of the primary device, the MIR 2 and the MIR 3 were also used as primary devices, but no differences in the results were observed (data not shown). Therefore, in the present work the results are presented based on the MIR 1 as primary device.

2.3.2.1. Instrument-specific correction factors. Fifteen QC sample measurements obtained from each of the three instruments were used to investigate the differences in the data sets of the three spectrometers. In a correlation diagram, the absorbances of three different spectral regions (1: 3041 cm^{-1} - 2790 cm^{-1}; 2: 1787 cm^{-1} - 1624 cm^{-1}; 3: 1448 cm^{-1} - 601 cm^{-1}) of the primary instrument (MIR 1) (y intercept) were plotted against the absorbances of the other two instruments (x intercept), separately. The y intercept was set to zero and the slope a reflected the relationship between the instruments, i.e. the instrument-specific correction factor.

In order to increase the comparability of MIR spectra of the three instruments, the determined correction factors of the respective spectral ranges (1: 3041 cm^{-1} - 2790 cm^{-1}; 2: 1787 cm^{-1} - 1624 cm^{-1}; 3: 1448 cm^{-1} - 601 cm^{-1}) were multiplied with each variable (intensity) of the subrange of the MIR 2 and MIR 3.

2.3.2.2. Optimal data pre-processing. Several method combinations were tested for pre-processing of spectral data (internal validation) (Table S2 in the Supplementary material). Normalization (norm area = 1), 1st derivative Savitzky-Golay (centred 11 point window) and mean centering were selected.

2.3.2.3. Piecewise direct standardisation (PDS) - calibration transfer. In this study, five rapeseed and five pumpkin seed oil samples were chosen as standardization samples from the sample set according to Griffiths et al. [53] and a window size of 11 spectral points to standardize each instrument. For the selection of the standardization samples, the Kennard-Stone algorithm was used to represent the complete variation of spectral data in the sample set [54]. It is important to mention here that these selected samples are not used for PLS-DA model building, optimization and validation. The standardization samples were measured on the primary instrument (MIR 1) and on each instrument that needs to be adjusted (secondary instruments - MIR 2 and MIR 3) using the same standard operation procedure (SOP). For further theoretical mathematical explanations, the reader is referred to the

publications by Wang et al. [47, 48]. For data evaluation, we used the PLS toolbox version 7.0.3 (Eigenvector Research, Wenatchee, WA, USA) and Matlab version 7.11.0 R2010b (The MathWorks Inc., Natick, MA, USA) (also described in section 2.3), which includes options for automatic calibration transfer. The PDS calculation is based on the stdgen function (S1 in the Supplementary material).

2.3.3. Data evaluation based on chemometrics

2.3.3.1. Explorative data analysis – principal component analysis (PCA).

The unsupervised method PCA was used to investigate the variation between the data sets resulting from each of the three instruments. In this study, the calculation of PCA was based on the singular decomposition algorithm and an internal cross-validation (venetian blind algorithm) was performed. Hotelling's T^2 probability distribution as a Student's t distribution [55] at the 95% confidence interval and Q residuals were used to detect possible outliers. In this study, no outliers were detected.

PCAs were calculated containing three different data sets and preprocessing steps:

a) QC samples and the seed oil sample set (n = 84) for quality assurance reasons (see section 2.2.1),
b) combined, original seed oil sample set (MIR 1, MIR 2 and MIR 3; n = 222) (see section 2.3.1) and
c) combined, corrected seed oil sample set (MIR 1, MIR 2 and MIR 3; n = 222).

2.3.3.2. Model building, optimisation and validation - PLS-DA.

PLS–DA is a supervised classification technique, which combines the properties of PLS with the classification ability of discriminant analysis (DA) [49]. A sample is assigned to group A or B (or −1 and 1), where the groups are previously specified by a representative set of samples. The PLS-DA model was calculated by the straightforward implementation of a statistically inspired modification of the PLS (SIMPLS) algorithm. Jong et al. have already described the SIMPLS algorithms theoretically [56]. The number of latent variables (LV) was based on the calculation of the root mean square error of cross-validation (RMSECV). The optimal number of LVs was chosen by plotting the values of RMESCV against the number of LVs and selecting the first minimum to avoid an overfitting. This minimum represents the balance between information and spectral noise and reflects the optimal number of LV [57]. An internal cross-validation (CV) was carried out using the Venetian blind algorithm with ten data splits on the training set [58]. The parameters (sensitivity, specificity, accuracy, precision and classification error) and equations described in Table S3 in the Supplementary material were applied and calculated to verify the suitability of the PLS-DA model for the defined problem [59]. The sensitivity in these calculations described the percentage of correct classification of rapeseed oil samples as rapeseed oils.

To compare the results of the performance parameters, as well as LV of the PLS-DA model, the following procedure was chosen (Fig. 1). The two-class PLS-DA model was built and optimized with the training set of the original spectral data from MIR 1 (primary instrument) [60]. For this, the spectral data set of MIR 1 (n = 74) was divided into a training set (66%, n = 49 edible oil samples) and a test set (34%, n = 25 edible oil samples) using the Kennard-Stone (KS) algorithm [54] to prevent the bias resulting from manual data splitting. The test set was used to validate the model of MIR 1 spectral data (external validation).

Subsequently, the original and corrected spectral data from MIR 2 and 3 were considered respectively as prediction sets (same samples as for the test set, but measured on MIR 2 and MIR 3) for prediction into the optimized model, to avoid over-optimistic prediction results.

3. Results and discussion

3.1. Results of approaches without corrections

3.1.1. Spectral differences in the data sets

To illustrate the influence of the different MIR instruments on the spectra and the resulting variation within these spectra, a line plot of the superimposed MIR spectra (number of samples (n) = 3*84 of MIR 1, MIR 2 and MIR 3 data and number of repetitions of one sample (m) = 3) is shown in Fig. 2 with highlighted typical bands of the two edible oil varieties.

Fig. 2 shows that the main differences are based on the absorbance

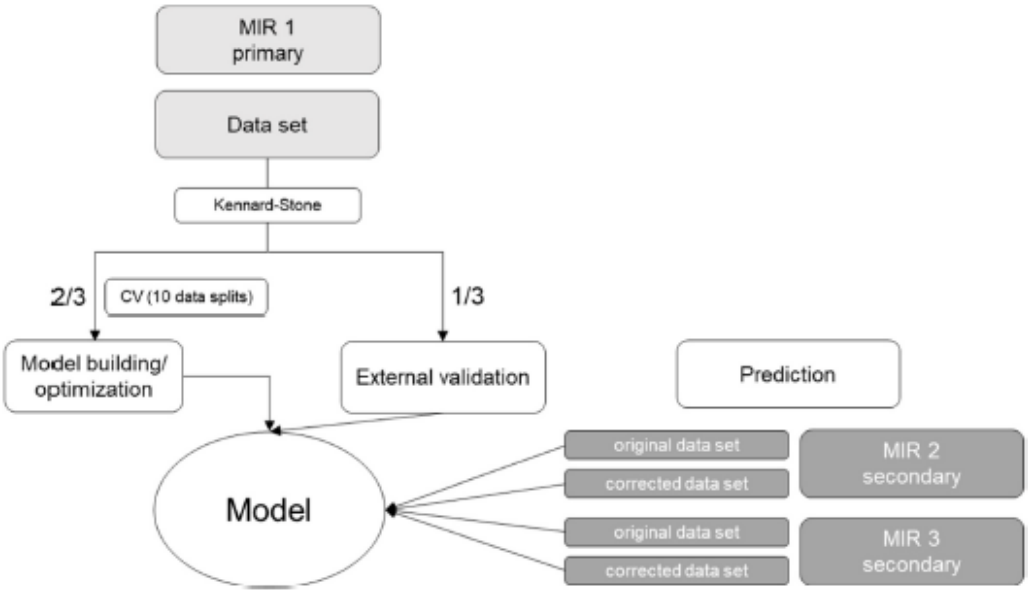

Fig. 1. Scheme of PLS-DA model building/optimization and internal validation (with training set of MIR 1), and external validation (with test set MIR 1) and prediction (prediction sets of MIR 2 and MIR 3), CV: internal cross-validation (10 data splits on training set).

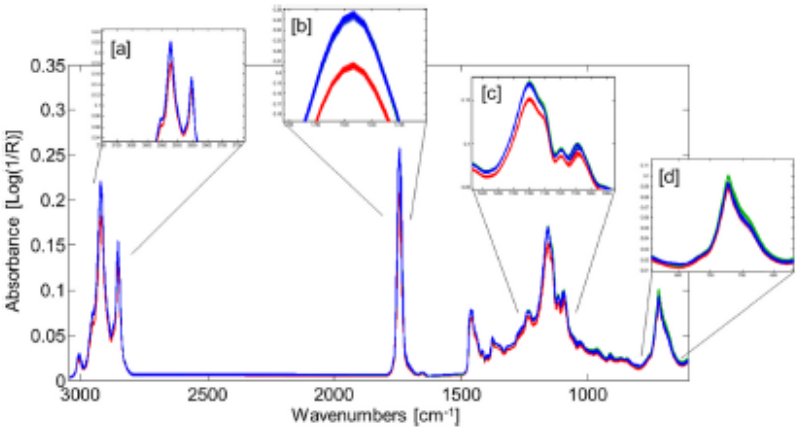

Fig. 2. Line plot of MIR spectra of the edible seed oil sample set (n = 3*84, m = 3) recorded using MIR 1 (red line), MIR 2 (green line) and MIR 3 (blue line); region [a]: at 2925 cm^{-1} and 2854 cm^{-1} (symmetric and asymmetric stretching vibration of the aliphatic CH_2 group); region [b]: at 1746 cm^{-1} (ester carbonyl functional group of the triglycerides); region [c]: e.g. band at 1119 cm^{-1} (associated with stretching vibration of the C-O ester groups) and band at 967 cm^{-1} (associated with bending vibrations of CH functional groups of isolated *trans*-olefins); region [d]: at 720 cm^{-1} (overlapping rocking vibration of CH_2 groups and out-of-plane bending vibration of *cis*-olefins) [43,44]. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

intensity: spectral data from MIR 2 and 3 show higher absorbance than MIR 1 (red line) over the entire spectrum. Presumably, these differences in absorbances are due to the different running times of the sources of the MIR instruments. The assumption that the absorbances of radiation decrease as the age of the radiation source increases is described in literature [24,61].

Further, no shifts in the wavenumbers associated with certain functional groups were apparent between the measurements using the three different MIR spectrometers. Except for the 720 cm^{-1} region, the absorbance of MIR 3 is higher than the absorbance of MIR 2. This band is attributed to the overlapping of the CH_2 rocking vibration and the out-of-plane vibration of *cis*-disubstituted olefins [62]. A decrease of the absorbance in this range indicates conjugation and *cis-trans* isomerization of double bonds and thus an oxidation of the samples. However, oxidation can be excluded in the presented case because the reactions described would also be accompanied by a decrease in absorbance at 3006 cm^{-1}, an increase in absorbance at 987 cm^{-1} and 967 cm^{-1} as well as the presence of bands at 3400 cm^{-1} and 1711 cm^{-1} [44,63], which were not observed. Thus, since oxidation of the samples can be excluded (also due to the short interval between measurements), the differences in the data sets are not only caused by the aging of the IR radiation source, but may also be attributed to different instrument designs due to the use of a different MIR manufacturer.

3.1.2. Explorative data analysis with original data from the three instruments

Before investigating whether the observed absorbance differences of the spectral data affect the multivariate data analysis, a PCA was performed using the QC samples and the sample set (rapeseed and pumpkin seed oil samples of each MIR instrument). Within the sample set of each

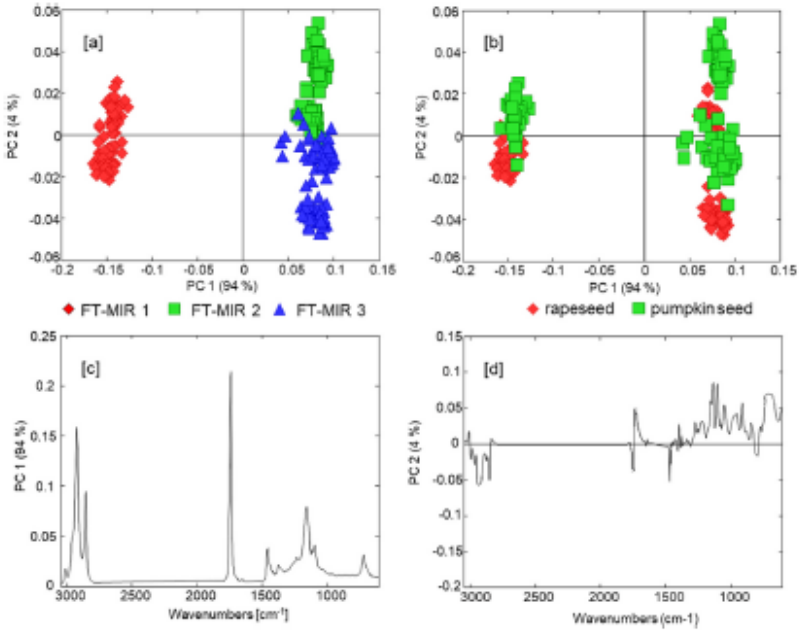

Fig. 3. PCA scores plot (PC 1; PC 2) of 40 rapeseed and 44 pumpkin seed oil samples (MIR original spectral data, see 2.3.1) recorded at MIR 1 (red diamonds), 2 (green squares) and 3 (blue triangle), respectively; [a] coloured by instrument, [b] coloured by seed oil variety; [c] Loading plot of PC 1; [d] Loading plot of PC 2. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

MIR spectrometer, the QC samples formed a separate group, which was visible in the respective PCA score plots for each instrument and no outliers (QC samples) or time- or analytically dependent drifts from inter-day variation as well as intra-day variation could be identified. Subsequently, a PCA was calculated based on the combined data set (MIR 1, 2 and 3) of rapeseed and pumpkin seed oil (Fig. 3).

In Fig. 3a (original data set - see section 2.3.1), three main clusters can be identified. Along PC 1, there is a clustering of MIR 1 vs. MIR 2 and 3 (94% of the variance explained). The data sets of MIR 2 and 3 cluster along PC 2 with 4% of the variance explained. Considering the loading plots (Fig. 3c) for PC 1, it is obvious that PC 1 is described in particular by the difference in the absorbances of the spectra. Within the cluster of each instrument, there is a similar grouping of the samples along PC 2 according to the seed oil variety (Fig. 3b). The rapeseed oil samples are always the upper cluster of each instrument. According to the loading plot (Fig. 3d), the clustering of the two seed oil varieties along PC 2 is caused by absorbance differences in the range of 1050 cm^{-1} - 800 cm^{-1} (high loadings), a region associated with saturated fatty acids. This difference between the two seed oil varieties was described by Beyzi et al. [42,45]. It can be concluded that, based on the descriptions, there is a greater influence on the variance from the difference between instruments than from the difference between seeds.

3.1.3. Assessment of PLS-DA results with original spectral data from the three instruments

The advantage of classification approaches compared with explorative data analysis is that performance parameters such as sensitivity, specificity, classification error or accuracy can be calculated and used for comparing the prediction results of original and corrected spectral data. Comparability of spectral data was assessed by predicting the data (measured with one instrument) using a developed model (with spectral data from another instrument).

As expected, the two-class PLS-DA model (rapeseed oil vs. pumpkin seed oil), built and optimized with spectral data from one spectrometer (MIR 1), achieved a prediction rate of 100% (classification error 0%) and showed no doubtful sample predictions for internal and external validation.

The model developed with MIR 1 spectral data was then used to predict data from the other instruments. In this step, the MIR 2 and 3 data sets were each used individually as prediction set and the PLS-DA results were compared. It should be emphasized that for the prediction of the prediction set (MIR 2 and MIR 3 data) exactly the same samples were selected as for the external validation with the test set of MIR 1, only measured with two different instruments. The calculated sensitivity was 0% for MIR 3 data, which means, that no rapeseed oil sample was identified as such: all were identified as pumpkin seed oil. A sensitivity of 20% was determined for the MIR 2 data (Table S4 in the Supplementary material). In contrast to the external validation with test set of MIR 1, the classification errors for prediction set were also quite high, with 40% for MIR 2 and 50% for MIR 3 (classification error of external validation with test set of MIR 1: 0%). Accordingly, less samples were correctly predicted with the prediction set than in the external validation with the test set of MIR 1, which was to be expected due to the differences in absorbance of the three instruments. These results show that the original spectral data cannot be used in a common database without further data handling. This highlights the need for suitable correction approaches to reduce the instrument-related variation in the data sets.

3.2. Results with correction approaches

The following parameters were chosen for each approach to correct the data from MIR 2 and 3:

3.2.1. Instrument-specific correction factors

For the use of one correction factor (based on 15 measurements of the QC sample), the coefficient of determination (R^2) was calculated to be 0.9987 for MIR 2 and 0.9970 for MIR 3 (Fig. S1 and S2 in the Supplementary material). A linear correlation was determined by calculating Pearson and Spearman coefficients (p-value <0.05), which are intended to detect a linear relationship between two variables. Nevertheless, from an absorbance of 0.15 for MIR 2 and 0.12 for MIR 3, an increasing one-sided deviation of the data points from the linear regression line could be observed. In addition, after evaluation by PCA and plotting of the loadings with one factor-corrected data, it was demonstrated that the deviating asymmetric valence vibration band of the CH_2 groups had a major influence on the first principal components (data not shown). Therefore, the bands of the asymmetric valence vibrations of the CH_2 groups (at 2925 cm^{-1}), as well as the valence vibration band of the esters (at 1746 cm^{-1}) were not ideally represented by the linear regression. In addition, the PLS-DA results showed that the use of only one calculated correction factor for each primary-secondary combination did not improve the classification results (especially for MIR 3 data) compared to the original data (Table S5 in the Supplementary material). In order to reduce this weighting of the bands with higher absorbance, the spectra were divided into different ranges. These were chosen so that the R^2 values were about 0.999 (criteria: $R^2 > 0.999$). Thus, the following three absorbance regions were determined: 1: 3041 cm^{-1} - 2790 cm^{-1}; 2: 1787 cm^{-1} - 1624 cm^{-1}; 3: 1448 cm^{-1} - 601 cm^{-1} (Table 1). As a result, the data points of the higher absorbance were less represented and their influence on the calculation of the factors was reduced.

This procedure was also performed using three different sunflower ($n = 3$, $m = 3$) and linseed oil samples ($n = 3$, $m = 3$), to verify that the correction factors could be applied independently of variety. The slopes (correction factors for each spectral region), determined by the QC sample and the two other seed oil samples were ≤ 0.01, and R^2 was greater than 0.999 for all evaluations.

3.2.2. Optimal data pre-processing

For each pre-processing combination, PLS-DA models were calculated and the optimal pre-processing was identified (in the internal validation) based on the following performance parameters: sensitivity, specificity, accuracy, precision; number of LV and the classification errors were also calculated. The best PLS-DA (including LV) results were obtained using the pre-processing steps normalization (norm area = 1), 1st derivative Savitzky-Golay (centred 11 point window), followed by mean centering. In addition, to get an overall view of the generated models and to check the application of the established models, the data from MIR 2 and MIR 3 were predicted separately into each model with different PP and also the performance parameters and LV were determined (Table S6 in the Supplementary material).

3.2.3. Piecewise direct standardisation (PDS) – calibration transfer

According to literature, there are different optimization possibilities [64,65]. In the present study, different window sizes (7–13) with different spectral points were tested for the calculation of the regression coefficients. For the question investigated in this study, a window size of 11 spectral points was the most appropriate with respect to the PLS-DA results (best results for sensitivity, specificity and classification error for the internal validation (data not shown).

Table 1
Calculation of instrument-specific correction factors and R^2 for MIR 2 and 3 in the selected three regions of the QC sample ($n = 15$, $m = 3$).

Instrument	Spectral region 1 (3041 cm^{-1} – 2790 cm^{-1})		Spectral region 2 (1787 cm^{-1} – 1624 cm^{-1})		Spectral region 3 (1448 cm^{-1} – 601 cm^{-1})	
	factor	R^2	factor	R^2	factor	R^2
MIR 2	0.8611	0.999	0.8264	0.999	0.9023	0.999
MIR 3	0.8452	0.999	0.8214	0.999	0.9229	0.999

3.2.4. PCA with original spectral data from MIR 1 and corrected spectral data from MIR 2 and 3

Fig. 4 illustrates the calculated PCA scores plots (PC 1/PC 2) of the combined data set (with original spectral data from MIR 1 and the corrected spectral data from MIR 2 and 3: see 2.3.2) of pumpkin seed and rapeseed oil samples from the three instruments. The PCA score plot after correcting the spectral data from MIR 2 and 3 with the instrument-specific correction factors (see 2.3.2) in Fig. 4 (1a/b) indicates that the main cause of cluster formation along PC 1 (with 42% of explained variance) is still the instrumental difference, the seed difference is now described with 36% of explained variance along PC 2. In addition, it can be recognized that especially the data points of MIR 3 (made by a different manufacturer than MIR 1 and 2) group along PC 1 away from the clusters of the other two instruments. As described previously in 3.1.1, this suggests that the differences in the data sets are also related to device settings or similar device-dependent parameters (not only for the entire spectrum but for specific areas, too). Accordingly, the differences, which are described in particular by specific areas in the spectrum (from an absorbance of 0.12), cannot be completely compensated for by the correction factors.

Compared with the results from using the instrument-specific correction factors, the differences in the data sets seem to be reduced to a greater extent and clustering according to the seed variety (PC 2) becomes clearer using the optimal pre-processing of original spectral data. Pre-processing provides the use of sample information to reduce measurement-related effects (e.g., differences in radiation source intensities), but information can also be lost or altered by overfitting [52]. Nevertheless, in this study, the observed interference effects were not

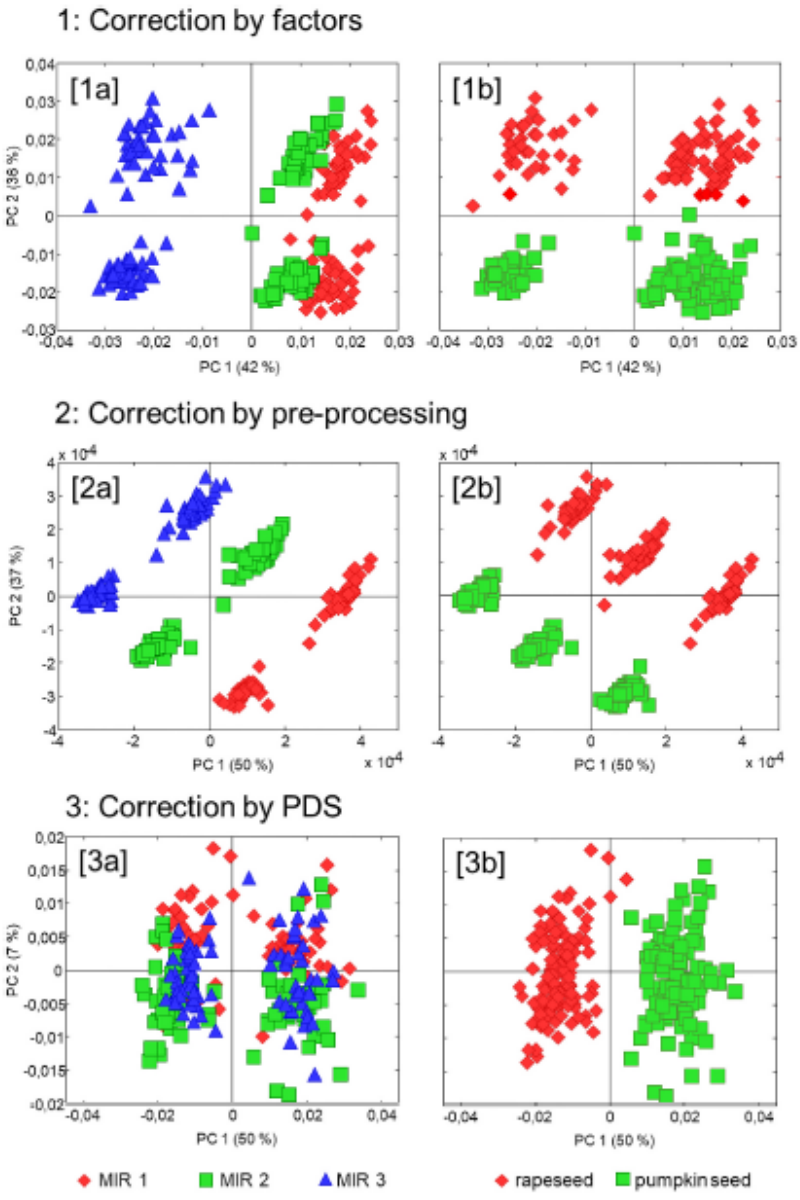

Fig. 4. Scores Plots (PC 1; PC 2) of PCA for pumpkin (n = 39) and rapeseed oil (n = 35) sample set, measured on MIR 1 (red diamonds), MIR 2 (green squares) and MIR 3 (blue triangles); [1a/b] spectral data from MIR 2 and 3 corrected by three factors (mean centering as pre-processing); [2a/b] spectral data from MIR 2 and 3 corrected by optimal pre-processing (normalization (norm area = 1), 1st derivative Savitzky-Golay (centred 11 point window) and mean centering); [3a/b] spectral data from MIR 2 and 3 corrected by piecewise direct standardization (PDS) (mean centering as pre-processing). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

completely eliminated by pre-processing, as is illustrated in Fig. 2a/b (groupings by instrument). This could be due to fact that the spectrum does not consistently reveal lower or higher absorbances over the entire spectrum.

The PCA score plot based on the spectral data corrected by PDS is presented in Fig. 4 (3a/b). Here, two main clusters are visible along PC 1 (50% of the variance explained), which are described by the seed oil variety. Only a marginal influence of the instruments can be recognized (points of MIR 1 grouped further up along PC 2). The other PCs and, consequently, the explained variance are influenced in particular by the specific differences between the varieties.

In summary, the correction with PDS revealed that the main PCs are described by the differences of seed oil variety and not of instrument.

3.2.5. Assessment of PLS-DA results with corrected spectral data from MIR 2 and 3

The results of the performance parameters were used to investigate whether the correction approaches improve the comparability of the spectra. In particular, the results of the prediction of the prediction set using the original (3.1.3) and the corrected spectral data (Table 2) were compared.

The results demonstrate that with all three correction approaches, all rapeseed oil and pumpkin seed oil samples were assigned to the correct class (classification error 0%). Considering that the performance parameters provide the same results, all three approaches are reviewed below for their advantages and disadvantages regarding their use in non-targeted analysis and the establishment of a common database.

3.3. General discussion of the selected correction and transfer approaches

Regarding the method's routine use, the optimal solution to minimize spectral differences would be an approach that requires little time and minimal laboratory/mathematical effort. This is equally relevant to the development of a common spectral database. In general, when building such a database, it is important to consider what the goal and focus should be (e.g., only for specific matrices/questions, etc.) of the database [19]. Furthermore, two aspects need to be addressed in advance. First, it is necessary to define the structure of the database. Specifically, it must be determined in the preliminary stages whether the database will be operated by one laboratory, which performs the validation of new laboratories (primary-secondary system), thereby operating the spectral corrections and subsequently updating the database, or whether laboratories provide their spectral data without using a defined primary laboratory. Second, it must be defined what should be stored in the database. This could be raw spectral data, corrected spectral data, as well as mathematical data evaluation models. It has been proposed in this study that correction approaches are required to enable comparability of spectral data from different laboratories and, consequently, to make it possible to use the spectral data in a common database for MIR analysis of edible oil samples. The selected correction approach must be checked for its suitability regarding the structure and storage of spectral data or models.

Table 2
Classification results of PLS-DA predictions, with MIR 1 spectral data used for model building, and corrected spectral data from MIR 2 and 3 used as prediction set.

		Instrumental-specific correction factors		Pre-processing		PDS	
		MIR 2	MIR 3	MIR 2	MIR 3	MIR 2	MIR 3
Results (with corrected MIR 2 and MIR 3 data	Sensitivity [%]	100		100		100	
	Specificity [%]	100		100		100	
	Classification error [%]	0		0		0	

PDS: Piecewise direct standardization.

In the IR range, the authors are not aware of any publication that deals with the improvement of the comparability of spectra by means of factor calculation. Akoka et al. described a method for quantitative NMR spectroscopy in the field of spectrum optimization by means of an instrument-specific factor [27]. For this, the quotient of the ERETIC factors, each calculated from values (selected signals, NMR-specific parameters) from two instruments, was used as a correction factor. However, this method focuses on the evaluation of a single or a few signals in the spectra. In the present study, the calculation was based on a QC sample analyzed with all instruments as well. This offers the advantage that this sample can be used simultaneously as a reference sample and as a quality control sample to include quality assurance measures. Thus, no additional samples have to be measured. Moreover, changes in the instrument will become apparent, which can be communicated from the laboratory to the reference laboratory so that the factors can be recalculated. Within the scope of this study, it was tested whether other edible oils (sunflower and linseed oil samples) have an influence on the determination of the correction factor. It seems reasonable to assume that the calculation can be performed independently of the matrix. However, further investigations must be carried out for this purpose. It became apparent in the present PLS-DA evaluation that one factor is not sufficient to ensure comparability between the spectra, because no improvement in sensitivity and specificity (0%, respectively) could be achieved. Accordingly, no sample was assigned to the correct class. In addition, factors for other spectral ranges were calculated, which showed an $R^2 < 0.999$ and also did not improve the performance parameters (data not shown). Therefore, the ranges were selected to fulfill the criterion $R^2 > 0.999$. This procedure must be calculated for each new device added to the database, which can be time-consuming if no automated procedure is available. If this automated procedure is performed by the laboratory providing the primary instrument, the corrected spectral data can be uploaded to the database by this laboratory without further time-consuming activity for other laboratories. It will be possible to integrate new samples into the database in this way. However, a crucial step in the calculation of the correction factors is the selection of the spectral ranges and the number of measurements of the QC sample that are included in the calculation of the factors. Especially regarding the latter aspect, it is important to be aware of the fact that the R^2 value depends on the sample size. Therefore, in order to apply this approach in practice, to ensure the comparability of spectral data and thus to take a step towards harmonization of non-targeted methods, further investigations should be carried out regarding the selection of spectral ranges as well as the sample size. In addition, it must be taken into account that a primary instrument becomes unstable over time or is not operational anymore. Therefore, it is essential to define one or more additional instruments as primary instruments for standardization and adaptation purposes, for instance, in accordance with Grelet et al. [39].

Holland et al. successfully demonstrated in 1997 that absorbance differences of spectra measured on two MIR instruments can be an optimized pre-processing. Correct prediction rates of 100% were obtained for a discriminant analysis model based on five PCs for external validation when the model of one spectrometer was transferred to the other. However, the authors noted that for this specific example, the first PC was described by the spectral differences caused by the devices. In the presented study, classification rates of 100% were also achieved. In comparison with the study of Holland et al., additional parameters were included in the assessment of the suitability of the correction approaches such as sensitivity and specificity, calculated for each validation step. In addition, Holland et al. showed that different correction rates were obtained with changes in the number of PCs used to build the model. The authors indicated that a different type of pre-processing and number of

PCs would be required for a different authenticity problem. This illustrates that the pre-processing-based approach is usually optimized for a specific question and thus still represents an isolated solution and suggests that optimizing the pre-processing does not ensure comparability between instruments. This would have to be tested individually. In addition, the entire data set or the model has to be recalculated when new samples are added to the database. Accordingly, this approach is limited for building a common database.

In the PDS-based approach, the primary-secondary system is used as in the calculation of the factors. However, in contrast to the determination of the factors, a certain number of standardization samples must be measured in each laboratory; the measurement of one QC sample is not sufficient. The selection of these standardization samples before transfer parameters can be generated is of crucial importance in order to prevent poor standardization [23]. The number and type of standardization samples have already been discussed in many publications, although no specific number has been fixed. However, two points should be considered in the selection. The samples should be very similar to the matrix under investigation, but should also cover the full variability of the matrix [30]. The greater the complexity of the authenticity question (more than two classes), the more standardization samples need to be measured to include the complete variability of the different spectra. In this study, the standardization samples were selected according to KS algorithm for each class and consequently, the complete variability of the data set was represented in the standardization sample set. Subsequently, standardization samples must be selected for each class/sample type and specifically for each authenticity question. Accordingly, this approach is not independent of the authenticity question. However, a major advantage of this approach is that raw spectral data and models can be stored in a common database (prerequisite measurement of standardization samples). Numerous studies have demonstrated the applicability of PDS [38,39,47]. These examples also show the benefits of the approach with respect to the use of the models over a longer period of time and the inclusion of new samples. However, it is also clear from the investigations described in these studies that the approach is likewise limited to a specific product.

The listed advantages and disadvantages illustrate that in order to enable an application/transfer into routine, in addition to further investigations, the structure and aim of a database need to be defined in advance. Regarding the construction of a common database (using the primary-secondary system), the two approaches using correction factors and PDS are the most promising in this study. In summary, a laboratory network with one primary instrument (or several primary instruments) appears to be particularly advantageous if one institution monitors the database, organizes quality assurance measures centrally for the entire network and performs the calculations automatically. In such a case, all steps of the measurement including data processing, determination of the factor/coefficient and database reconciliation should be precisely recorded in sets of rules which are freely accessible to all participants.

4. Conclusion

An essential step towards harmonization in non-targeted analysis is the development of approaches, which ensure the comparability of spectral data obtained with different instruments (using the same technique). In this study, different approaches (instrument-specific correction factor, pre-processing and PDS) were proposed to ensure the comparability of MIR spectra, and their advantages and disadvantages for the application of a common database were discussed. Differences in the intensities of absorbance of three MIR spectrometers were observed. These differences influenced the results of the PCA and the two-class PLS-DA model leading to low sensitivity (with prediction set) without application of corrections (20% for MIR 2 and 0% for MIR 3). This means that, in order to develop a robust model, measurements from different instruments cannot be combined without adjustments using mathematical correction approaches. The three investigated correction approaches resulted in an improvement of values of the performance parameters. The authors prefer correction by PDS for the spectral data presented in this study. In further studies, other calibration transfer approaches could be tested, e.g., wavelet transform-based standardization technique or orthogonal signal correction, to verify if these approaches are also suitable for the application of classification models. In addition, subsequent studies should be conducted involving additional manufacturers of MIR instruments and also other pre-processing steps with different settings (like window size).

The comparison of the approaches on a specific example demonstrated that, before establishing a common database, it is important to define what question needs to be addressed and what should be stored in the database.

CRediT authorship contribution statement

Carolin Lörchner: Investigation, Formal analysis, Writing – original draft. **Carsten Fauhl-Hassek:** Project administration, Supervision, Writing – review & editing. **Marcus A. Glomb:** Supervision, Writing – review & editing. **Vincent Baeten:** Conceptualization, Writing – review & editing. **Juan A. Fernández Pierna:** Formal analysis, Writing – review & editing. **Susanne Esslinger:** Conceptualization, Writing – review & editing.

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.

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemolab.2023.104904.

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5 Zusammenfassung und Einordnung der Forschungsergebnisse

Im Jahr 2016 wurde vom Bundesinstitut für Risikobewertung (BfR) ein Symposium ausgerichtet, welches sich erstmalig und bis heute einzigartig mit dem Thema „Standardisierung von nicht-zielgerichteten Analyseverfahren für die Authentizitätsprüfung von Lebensmitteln“ beschäftigt hat. Auf der Website des BfR wurde hierzu veröffentlicht: „Voraussetzung für die Anwendung dieser neuartigen nicht-zielgerichteten Verfahren in der Routine ist die Validität der Ergebnisse und ihre Vergleichbarkeit bei der Anwendung in verschiedenen Laboratorien. Hierzu fehlen bislang wichtige Grundlagen wie standardisierte und valide Analyseverfahren inklusive der notwendigen statistischen Auswertung sowie gemeinsam zugängliche und nutzbare Datenbanken.“ (BfR, 2016). In den darauffolgenden Jahren wurden eine Vielzahl von *Reviews* veröffentlicht, die diese Standardisierung/Harmonisierung in der nicht-zielgerichteten Analytik fordern, u. a. im Bereich der Validierung (Methodenvalidierung, Modellvalidierung einschließlich interner und externer Validierung, analytische Validierung) (Cavanna et al., 2018; Gallo et al., 2020; Gao et al., 2019; McGrath et al., 2018).

Die vorliegende Dissertation stellt sich in den folgenden Kapiteln diesen Herausforderungen und beschreibt Lösungsansätze. Dies erfolgt am Beispiel der Matrix Speiseöle, analysiert mittels spektroskopischer Techniken (FT-MIR-, FT-Raman- und ^{1}H-NMR-Spektroskopie) und anschließender multivariater Datenauswertung.

5.1 Harmonisierung von Validierungskonzepten in der Authentizitätsprüfung (Publikation A)

Die Verfälschung von hochwertigem mit günstigem Speiseöl ist eine gängige, dem *Food Fraud* zuzuordnende Praktik, die der Erzielung eines hohen finanziellen Gewinns dient.

In der vorliegenden Dissertation wurde exemplarisch das Potenzial dreier spektroskopischer Verfahren (FT-MIR-, FT-Raman- und ^{1}H-NMR-Spektroskopie) zur Entdeckung der Verfälschung von kaltgepresstem Kürbiskernöl mit raffiniertem Rapsöl untersucht, bewertet und verglichen. Hierbei stand insbesondere die Eignung als Schnellverfahren, um eine Verfälschung frühzeitig identifizieren zu können, im Vordergrund. Die Datenauswertung beruhte dabei auf einer multivariaten, quantitativen Regressionsanalyse (PLS-R). Zudem wurde untersucht, inwieweit Validierungskonzepte und *performance* Parameter in der Literatur Anwendung fanden und inwieweit es einer Harmonisierung dieser Konzepte bedarf.

Eine PLS-R wurde durchgeführt, um das geschätzte *Minimum Detection Level* (MDL) (2 x RMSEP) für die Quantifizierung von raffiniertem Rapsöl in kaltgepresstem Kürbiskernöl für jedes Analyseverfahren unter Verwendung eines unabhängigen zusätzlichen Testdatensatzes (*system challenge,* siehe 2.2.4.4) zu bestimmen, entsprechend der Beschreibung von Downey und Kelly (Downey & Kelly, 2004). Zudem wurde neben der Berechnung des MDL ein weiterer Schwerpunkt auf die Bewertung und den Vergleich anderer Modellparameter wie RMSE- und R^2-Werte gelegt, die mit den drei spektroskopischen Techniken ermittelt wurden. Es zeigte sich, dass alle drei Techniken geeignet waren, Verfälschungen von raffiniertem Rapsöl in Kürbiskernöl unter 10 % nachzuweisen. Beim vorliegenden Beispiel handelt es sich eher um eine zielgerichtete Authentizitätsfragestellung, da die Detektion einer

bekannten Verfälschung (raffiniertes Rapsöl) im Vordergrund stand (McGrath et al., 2018). Nichtsdestotrotz wurde zur Auswertung das gesamte Spektrum multivariat ausgewertet, was eine Modellvalidierung erforderlich macht. Dieses Beispiel sollte verdeutlichen, dass insbesondere bei der multivariaten Quantifizierung verschiedenste Parameter in die Bewertung mit einbezogen werden müssen, die Validierung sich jedoch nicht von einer nicht-zielgerichteten Modellvalidierung unterscheidet.

Um die PLS-R-Parameter einordnen und bewerten zu können, wurden die generierten Ergebnisse mit in der Literatur beschriebenen Ansätzen (Berechnungen, Bewertungsparameter, Modellerstellung) für ähnliche Authentizitätsfragestellungen und spektroskopische Verfahren verglichen (Tabelle 3). Die in der vorliegenden Dissertation ermittelten *performance* Parameter (RMSEP, RMSECV, MDL) sind im Wesentlichen mit den in der Literatur beschriebenen Werten (falls vorhanden) vergleichbar bzw. liegen in einem ähnlichen Bereich.

Tabelle 3: Ausgewählte Beispiele aus der Literatur hinsichtlich der nicht-zielgerichteten Authentizitätsprüfung von Speiseölen und der verwendeten *performance* Parameter zur Beurteilung der multivariaten Regressionsmodelle.

Technik	**Frage-stellung**	**Modell**	***Performance* Parameter**	**Validierungs-konzept**	**Literatur**
NIR	Verfälschung von Kürbiskernöl mit raffiniertem Sonnenblumenöl	OPLS-R	RMSEE und RMSECV (2,298 bis 6,668)	nein	(Balbino et al., 2022)
Raman (tragbares Gerät)	Verfälschung von Kürbiskernöl mit Sonnenblumenöl	PLS-R	Vorhersage-gleichung	nein	(Becze & Simedru, 2020)
MIR und Raman	Verfälschung von kaltgepresstem Rapsöl mit raffiniertem Sonnenblumen- und Rapsöl	PLS-R	$R^2_{Pred.}$ und MDL-Werte ($R^2_{Pred.}$: 0,99; MDL: 9 % w/w)	Verwendung von Kalibrier- sowie Validierungssatz	(McDowell et al., 2018)
^{1}H-NMR	Verfälschung von kaltgepresstem Rapsöl mit raffiniertem Sonnenblumen- und Rapsöl	PLS-R	$R^2_{Pred.}$ und MDL-Werte ($R^2_{Pred.}$: 0,99; MDL: 8 % w/w)	Verwendung von Kalibrier- sowie Validierungssatz	(McDowell et al., 2018)
^{1}H-NMR	Verfälschung von Olivenöl mit verschiedenen Pflanzenölen (Sonnenblumenöl, Haselnussöl usw.)	PLS-R	RMSEP-Werte (0,32 % bis 3,4 %) Nachweisgrenzen (2 % und 5 %) (je nach Art der Verfälschung)	interne Validierung mittels *leave-one-out* Algorithmus sowie externe Validierung	(Alonso-Salces et al., 2022)

NIR = *Nearinfrared*; MIR = *Midinfrared*; NMR = *Nuclear Magnetic Resonance*; PLS-R = *Partial Least Squares-Regression*; OPLS-R = *orthogonal projection on latent structures*-Regression; RMSEP = *Root Mean Square Error of Prediction*; RMSEE = *Root Mean Square Error of Estimation*; MDL = *Minimum Detection Level*; $R^2_{Pred.}$ = Bestimmtheitskoeffizient *for Prediction*.

Bei der Bewertung der Ergebnisse sind verschiedene Punkte zu berücksichtigen, beispielsweise sind einige *performance* Parameter wie der MDL abhängig von der Authentizitätsfragestellung zu betrachten. Bei der Verfälschung eines Speiseöls durch ein anderes Speiseöl ist z. B. die Ähnlichkeit in der Zusammensetzung relevant. Dies bedeutet, dass bei großen Unterschieden u. a. in der Fettsäurezusammensetzung die Werte für MDL niedriger sein können und somit geringere Verfälschungsgrade nachgewiesen werden können als bei Speiseölen mit einer ähnlichen Fettsäurezusammensetzung, wo die Werte für MDL ggf. höher sind und daher erst höhere Verfälschungsgrade nachweisbar sind. Verdeutlicht wird dies u. a. durch das Beispiel von McDowell *et. al.* (McDowell et al., 2019; McDowell et al., 2018). Die Autoren untersuchten die Verfälschung von kaltgepresstem Rapsöl mit raffinierten Sonnenblumen- sowie Rapsöl. Aufgrund dessen, dass kaltgepresstes und raffiniertes Rapsöl eine ähnliche Fettsäurezusammensetzung aufweisen, wurden höhere MDL-Ergebnisse (Raman 22 % und MIR 64 %) erzielt als bei Verwendung von raffiniertem Sonnenblumenöl als Verfälschungsmittel (Raman 15 % und MIR 9 %).

Unabhängig von der Authentizitätsfragestellung sind jedoch Parameter wie RMSECV oder RMSEP. Diese Werte verdeutlichen die Qualität eines Modells (siehe Kapitel 2.2.4.4). Daher ist es notwendig, nicht nur MDL zu betrachten, sondern auch die genannten Modellparameter. Bei einem sehr niedrigen MDL, aber sehr hohem RMSECV muss überprüft werden, ob das Modell nicht zu „einfach“ aufgebaut wurde. Dies bedeutet, dass z. B. zu wenige Proben als Testdatensatz eingesetzt wurden, was auf ein *overfitting* hindeuten könnte (siehe Kapitel 2.2.4.4). Es ist daher wichtig, dass *performance* Parameter aus allen durchgeführten Validierungsschritten mit in die Bewertung einbezogen werden. In der vorliegenden Arbeit wurde das Konzept nach Riedl *et al.* angewendet (Riedl et al., 2015), da es aufgrund der Einbeziehung aller

wichtigen Parameter zur Modellvalidierung und zur Beurteilung eines Modells am umfassendsten ist (Brereton, 2009; Eriksson et al., 2006).

Die in Tabelle 3 aufgelisteten Studien zeigen zudem, dass es sehr unterschiedliche Ansätze für die quantitative Bestimmung von Verfälschungen in Speiseölen gibt. Die Verfahren unterscheiden sich in der Spektrenauswertung (z. B. Auswahl spezifischer, spektraler Bereiche oder *binning*), in der Art des Modells (z. B. PLS-R, OPLS), in der Berechnung der Nachweisgrenzen (LOD, MDL) oder auch in der Wahl der *performance* Parameter (so vorhanden), die zur Bewertung der mathematischen Modelle verwendet werden. Zudem werden auch unterschiedliche Begriffe für die gleiche Bedeutung eingesetzt, wie u. a. Kalibrierungs- und Validierungssatz vs. Trainings- und Testdatensatz. Dies verdeutlicht, dass in der nicht-zielgerichteten Authentizitätsprüfung bisher noch kein allgemein anerkanntes, harmonisiertes Konzept zur Modellbildung, -optimierung und -validierung existiert bzw. angewendet wird, was einen Vergleich der Studienergebnisse erschwert.

In der Literatur wurden bereits Validierungskonzepte (bezüglich Validierung der Modelle) z. B. von Riedl *et al.*, Alewijn *et al.*, Schönberger *et al.* oder Cavanna *et al.* vorgeschlagen (Alewijn et al., 2016; Cavanna et al., 2018; Riedl et al., 2015; Schönberger et al., 2015). Die Gemeinsamkeit in den Konzepten besteht darin, dass eine sogenannte interne sowie externe Validierung vorgeschlagen wird. Jedoch weisen diese ebenfalls Unterschiede u. a. in den Begrifflichkeiten auf. In den darauffolgenden Jahren wurden verschiedene Projekte zur Validierung nicht-zielgerichteter Analyseverfahren begonnen. Es wurde u. a. die Arbeitsgruppe CEN/TC 460 - *Food Authenticity* gegründet, welche sich mit allgemeinen Themen wie der Terminologie und den Definitionen sowie Validierungskonzepten in der nicht-zielgerichteten Analytik auseinandersetzt (CEN/TC 460/WG 5). Im Jahr 2019 wurde

zudem ein Leitfaden zur Entwicklung und Validierung dieser Analyseverfahren zum Nachweis von Verfälschungen veröffentlicht, jedoch fällt u. a. die quantitative Bestimmung einer spezifischen Verfälschung außerhalb des Geltungsbereiches dieser Leitlinie ("Appendix XVIII : USP 3 S (FCC 11) Guidance on Developing and Validating Non-Targeted Methods for Adulteration Detection," 2019). Ein weiterer Leitfaden „*Guide to NMR Method Development and Validation – Part I: Identification and Quantification*", welcher im Jahr 2023 aktualisiert wurde, beschreibt die quantitative Bestimmung von Analyten mittels NMR. Hierbei liegt der Fokus auf der Beschreibung allgemeiner Anforderungen wie u. a. der Entwicklung und Validierung univariater Quantifizierungsmethoden (Schönberger et al., 2023). Eine Beschreibung der Entwicklung und Validierung der multivariaten Datenanalyse wird in Teil II des Leitfadens „*Guide to NMR Method Development and Validation – Part II: Multivariate data analysis*" gegeben (Schönberger et al., 2015). Neben dem Vorschlag einer Validierungsstrategie unter Verwendung eines Kalibier- und Testdatensatzes, werden auch Validierungsparameter beschrieben, die zur Beurteilung eines Modells herangezogen werden sollten, wobei zwischen Klassifikationsmodellen und multivariaten Kalibriermodellen unterschieden wird. Zur Beurteilung z. B. von PLS-R-Modellen zählen Parameter wie die Unsicherheit der Modelle in Form von RMSEP-Werten, die Präzision oder auch die Selektivität. Eine *system challenge*, wie sie von Riedl *et al.* vorgeschlagen wurde (Riedl et al., 2015), wird auch in diesem Leitfaden nicht beschrieben. In dem 2019 veröffentlichten USP Leitfaden „*Guideline for the development and validation of non-targeted methods*" wird ebenfalls ein Validierungskonzept vorgeschlagen, wobei auch hier unterschiedliche Begrifflichkeiten verwendet werden (u. a. Referenzdatensatz anstelle von Trainingsdatensatz) ("Appendix XVIII : USP 3 S (FCC 11) Guidance on Developing and Validating Non-Targeted Methods for Adulteration Detection," 2019).

Diese Vielzahl an wissenschaftlichen Publikationen und Leitlinien verdeutlichen, dass in den nächsten Jahren ein Fokus auf der Entwicklung eines harmonisierten Validierungskonzeptes (Modellvalidierung) gelegt werden muss, welcher von allen Arbeitsgruppen gleichermaßen herangezogen werden kann, damit Auswertungen im multivariaten Bereich vergleichbar beurteilt werden können. Je nach Modelltyp müssen andere Parameter zur Auswertung herangezogen werden, aber Definitionen oder auch die Validierungsstrategien könnten harmonisiert werden. Idealerweise sollte ein allgemeines Regelwerk z. B. im Rahmen einer international gültigen Norm erarbeitet werden, um so die Akzeptanz der Regelungen in der wissenschaftlichen Gemeinschaft zu stärken.

Neben dem Bedarf einer einheitlichen Terminologie und der vereinheitlichten Validierung der multivariaten Modelle, besteht auch die Notwendigkeit einer einheitlichen Methodenvalidierung in der nicht-zielgerichteten Analytik. Im Bereich der Validierung einer/eines Methode/Verfahrens (nicht jedoch der berechneten Modelle), gibt es eine Reihe von Validierungsleitlinien/Normen für die zielgerichtete Analytik, die grundsätzlich auch für die nicht-zielgerichtete Analytik gelten (DIN EN ISO/IEC 17025:2018-03, 2018; DIN ISO 5725, 1994; ICH, 2022). Sie beschreiben Kriterien, die für eine Validierung einer Methode oder eines Verfahrens entscheidend sind (u. a. Sensitivität, Linearität, Spezifität), können jedoch nicht eins zu eins angewendet werden, da in der nicht-zielgerichteten Analytik das gesamte Spektrum (bestehend aus mehreren hundert Variablen) für die Datenauswertung verwendet wird und die bestehenden Validierungsparameter nur für Einzelverbindungen berechnet werden können. Auch Nichani *et al.* beschrieben in ihrer 2023 veröffentlichten Studie, dass diese Aspekte immer noch einer Harmonisierung bedürfen (Nichani et al., 2023). Ein Harmonisierungsaspekt stellt u. a. die analytische Validierung nicht-zielgerichteter

Methoden dar, wie die Gewährleistung der Qualität der instrumentellen Leistung als eine Qualitätskontrollmaßnahme, welche im folgenden Kapitel detailliert erläutert wird.

5.2 Qualitätssicherung in der nicht-zielgerichteten Analytik (Publikation B)

Im Rahmen verschiedener Normen (DIN EN ISO/IEC 17025:2018-03, 2018; DIN ISO 7870-1:2019, 2021) sind Anforderungen an interne Qualitätskontrollmaßnahmen festgelegt. Ein Beispiel für eine solche Maßnahme ist die Verwendung einer QS-Probe, die der regelmäßigen (messtäglichen) Überprüfung des gesamten analytischen Verfahrens einschließlich der Probenvorbereitung sowie der Messung dient und eine schnelle Identifizierung von zeitlichen und/oder instrumentellen Trends oder Ausreißern ermöglicht (Esslinger et al., 2014). In der zielgerichteten Analytik werden Trends mit Hilfe von univariaten Kontrollkarten ermittelt. In den letzten Jahrzehnten wurden je nach Art der QS-Probe, d. h. je nachdem, ob es sich um ein zertifiziertes oder hauseigenes, internes Referenzmaterial handelt, unterschiedliche Kontrollkarten entwickelt (DIN ISO 7870-1:2019, 2021; DIN ISO 7870-2:2013, 2021). Die Gruppe um Shewhart und Deming war eine der ersten, die eine univariate Regelkarte auf der Grundlage des Mittelwerts ($\bar{x}$) von QS-Messungen in der Prozesskontrolle entwickelt hat (Shewhart & Deming, 1939). Die Autoren empfahlen, obere und untere Warngrenzen ($\bar{x}$ der Vorperiode ± 2-fache Standardabweichung) sowie Eingriffsgrenzen ($\bar{x}$ der Vorperiode ± 3-fache Standardabweichung) in die Kontrollkarten zu integrieren. Als Anhaltspunkte für systematische Abweichungen dienen diese Grenzwerte zur Überwachung der täglichen Schwankung einer einzelnen Variablen in einem analytischen Prozess (z. B. der bekannte Gehalt einer spezifischen Verbindung in der QS-Probe). Dieser Ansatz ist gängige Praxis in vielen Teilbereichen der analytischen Chemie, auch in der zielgerichteten Authentizitätsprüfung. In der

Informatik werden ähnliche Fragen im Zusammenhang mit dem Aspekt der Ausreißeridentifizierung behandelt. Hier beruhen die Ansätze auf sog. *outlier score*-basierten Modellen, welche je nach dem angewandten Algorithmus für jeden Datenpunkt (also für jede Messung) einen *outlier score* berechnet (Breunig et al., 2000; Chandola & Kumar, 2009; Kordos et al., 2010; Turlach, 1993). Dieser Wert beschreibt den Abstand einer Messung zum Zentrum eines Datensatzes und kann zur Interpretation der Messungen herangezogen werden.

Eine ähnliche Vorgehensweise existiert in der nicht-zielgerichteten Analytik bisher nicht bzw. lediglich für einzelne Signale. Im Gegensatz zur zielgerichteten Analytik ist die quantitative Bestimmung eines einzelnen Merkmals in einer QS-Probe in der nicht-zielgerichteten Analytik zur Qualitätskontrolle nicht ausreichend. Zur Auswertung der QS-Probe müsste das gesamte Spektrum herangezogen werden, um jegliche Änderungen im Spektrum feststellen zu können. Dies könnte ein Grund dafür sein, dass z. B. lediglich 30 % der Publikationen im Bereich der nicht-zielgerichteten, massenspektrometrischen Analytik die analytische Variabilität durch Verwendung von QS-Proben beschreiben (Cavanna et al., 2018).

In der vorliegenden Dissertation (**Publikation B**) wurde daher ein standardisiertes Verfahren zum Führen einer multivariaten Kontrollkarte anhand eines praktischen Beispiels entwickelt und publiziert, welches den Anforderungen und Vorschriften der Qualitätssicherung in der zielgerichteten Analytik entspricht. Hierzu wurde eine QS-Probe (raffiniertes Rapsöl) mittels FT-MIR-Spektroskopie analysiert. Der Datensatz wurde anschließend verwendet, um verschiedene mathematische Tests zur Erkennung von Ausreißern zu untersuchen (PCA, PCA in Kombination mit dem Hotellings T^2- und Q-Residuen sowie vier verschiedene *outlier score*-basierte Algorithmen). Die Praktikabilität der mathematischen Modelle wurde mit QS-

Messungen, die unter Nicht-Standardbedingungen (absichtlich erzeugte Ausreißer) analysiert wurden, überprüft.

Die Auswertung von QS-Proben in Anlehnung an Sangster *et al.* mittels PCA (Sangster et al., 2006) wurde bereits in einigen Publikationen, welche qualitätssichernde Maßnahmen beinhalten, eingesetzt (Ehlers et al., 2022; Horn et al., 2021). Es zeigte sich, dass die Vorgehensweise der Ausreißerdetektion mittels PCA für den in dieser Arbeit verwendeten Datensatz zu unempfindlich ist und Ausreißer nicht zuverlässig bestimmt werden konnten. Daher wurden im zweiten Teil der Untersuchungen auf Grundlage der Hotelling's T^2- und Q-Residuen Konfidenzintervalle, die den Warn- und Kontrollgrenzen (95,5 % und 99,7 %) entsprachen, als Entscheidungskriterien zur Ausreißererkennung festgelegt. Hier zeigte sich jedoch ebenfalls, dass dieser Ansatz für die Anwendung als Qualitätskontrollinstrument nicht ausreichend empfindlich war, da nicht alle absichtlich erzeugten Ausreißer als solche detektiert werden konnten.

In einem dritten Auswertungsansatz wurden deshalb vier verschiedene *outlier score*-basierte Algorithmen angewendet. Hierbei wurden in Analogie zur zielgerichteten Analytik (DIN ISO 7870-1:2019, 2021; DIN ISO 7870-2:2013, 2021) zunächst Warn- und Kontrollgrenzen auf Grundlage einer Vorperiode ermittelt.

Im Überblick ergibt sich, dass sich alle vier Algorithmen für die Erstellung einer multivariaten Kontrollkarte zur Identifizierung von Ausreißern und der Anzeige eines zeitlichen Trends eignen. Den Untersuchungsämtern für Lebensmittelüberwachung und Tiergesundheit Baden-Württemberg ist es bereits ebenfalls gelungen, für ein nicht-zielgerichtetes, NMR-basiertes Verfahren für die Differenzierung von Eiern aus biologischer bzw. konventioneller Haltung eine vollautomatisierte Qualitäts-kontrollkarte mittels MATLAB® zu entwickeln (CVUA, 2022). Die detaillierte Vorgehensweise wurde bisher nicht publiziert. Mit dem hier dargestellten Ansatz

wurden zum ersten Mal simplifizierte Varianten für Auswertestrategien zur Führung von Qualitätskontrollkarten in der nicht-zielgerichteten Analytik entwickelt. Dieser Ansatz kann als Grundlage für laborinterne, qualitätssichernde nicht-zielgerichtete Analysen von anderen Laboren herangezogen werden.

5.3 Gewährleistung der Vergleichbarkeit spektraler Daten (Publikation C)

Durch die Etablierung von harmonisierten/standardisierten Modell- und Methodenvalidierungskonzepten inklusive einer einheitlichen Terminologie sowie geeigneten Qualitätssicherungsmaßnahmen kann gewährleistet werden, dass Untersuchungsergebnisse valide und zuverlässig sind. Diese Aspekte sind nicht nur in Bezug auf die gerichtsfeste Anwendung nicht-zielgerichteter Analysemethoden zur Authentizitätsprüfung von großer Bedeutung, sondern auch um sicherzustellen, dass Datenbanken mit validen Daten/Modellen gespeist wurden. Dies ist insbesondere wichtig, wenn laborintern aufgebaute Datenbanken mit anderen Datenbanken anderer Labore fusioniert werden sollen bzw. Datenbanken von mehreren Laboren aufgebaut werden, um laborübergreifend zu agieren. Beim Aufbau einer gemeinsamen Datenbank ist wichtig zu überlegen, welches Ziel und welcher Schwerpunkt (z. B. nur für bestimmte Matrices/Fragen usw.) mit der Datenbank verfolgt werden soll (Donarski et al., 2019). Darüber hinaus müssen zwei Aspekte im Voraus berücksichtigt werden: Zunächst muss die Struktur der Datenbank festgelegt werden. Insbesondere muss im Vorfeld entschieden werden, ob die Datenbank von einem Labor weiterentwickelt und gepflegt wird, welches die Spektralkorrekturen vornimmt und anschließend die Datenbank aktualisiert. Eine Alternative wäre, dass die Labore ihre Spektraldaten bereitstellen, ohne ein bestimmtes Labor als Überwachungsinstitution zu nutzen. Darüber hinaus muss festgelegt werden, welche Informationen in der Datenbank

gespeichert werden sollen. Dies können sowohl die originalen Spektraldaten, korrigierte Spektraldaten als auch mathematische Datenauswertemodelle sein. Um die Zuverlässigkeit der Labore aufzuzeigen, schlugen Donarski *et al.* zusätzlich vor, Metadaten wie die Messparameter mit in die Datenbank zu integrieren (Donarski et al., 2019). Die Nutzung gemeinsamer Datenbanken hätte den Vorteil, dass durch die Arbeit diverser Labore u. a. mehr Spektren einer Lebens-/Futtermittelmatrix in einer Datenbank gespeichert werden können, die natürliche Variabilität dieser Matrix (geografische und botanische Herkunft, Erntejahr, Vegetation, etc.) besser abgebildet und somit ein Spektrenvergleich mit einer umfassenden Referenz möglich wird.

Eine Herausforderung bei der Anwendung einer gemeinsamen Datenbank stellt die Vergleichbarkeit der erzeugten Spektraldaten aus verschiedenen Laboren dar. Die Gewährleistung der Vergleichbarkeit ermöglicht eine genaue Authentifizierung einer Probe mit Hilfe von Datenbanken und gültigen multivariaten Modellen, die von einem anderen Labor erstellt wurden. Um spektrale Unterschiede zwischen Daten zu minimieren, die unter Verwendung desselben Messverfahrens mit verschiedenen Instrumenten erfasst wurden, sind in der Literatur bereits verschiedene Korrekturansätze diskutiert worden (Bouveresse & Massart, 1996; Ehlers et al., 2022; Feudale et al., 2002; Gallo et al., 2020; Workman, 2018).

In der vorliegenden Arbeit wurden drei verschiedene Korrekturansätze - i) PDS, ii) *pre-processing* der Daten (u. a. Normalisierung, Ableitungen) sowie iii) gerätespezifischer Korrekturfaktor gegenübergestellt. Dies wurde am Beispiel von Kürbiskernöl- und Rapsölproben untersucht, die mit unterschiedlichen Geräten (identische Geräte desselben Herstellers und MIR-Spektrometer verschiedener Hersteller) gemessen wurden. Zusammenfassend konnte gezeigt werden, dass die getesteten Korrekturansätze zur Optimierung der Vergleichbarkeit von Spektraldaten verwendet

werden können. Eine detaillierte Diskussion zur Prüfung der drei Ansätze auf ihre Vor- und Nachteile bezüglich der Verwendung in der nicht-zielgerichteten Analytik und die Einrichtung einer gemeinsamen, laborübergreifenden Datenbank ist in **Publikation C** aufgeführt.

Es gibt bereits Bestrebungen von amtlichen Untersuchungseinrichtungen eine laborübergreifende Weindatenbank im Bereich der nicht-zielgerichteten ^{1}H-NMR-Spektroskopie zu etablieren, jedoch befindet sich diese noch im Aufbau (Riedl, personal communication, 10.02.23). Um den Anforderungen der EU-Kontrollverordnung (Nr. 2017/625) gerecht zu werden (Verordnung (EU) 2017/625, 2017), wurde in Deutschland das NRZ Authent gegründet, das u. a. den Auftrag hat, eine nationale Datenbank mit Analyseergebnissen von authentischen Referenzproben aufzubauen und bereitzustellen (Verordnung (EU) 2017/625, 2017; MRI, 2021). Auch diese Datenbanken befinden sich erst noch im Aufbau. Wie die Vergleichbarkeit von spektralen Daten adressiert wird, ist wissenschaftlich nicht beschrieben bzw. publiziert.

Durch die Ergebnisse in Publikation C konnte ein wesentlicher Beitrag in Richtung Harmonisierung nicht-zielgerichteter Verfahren geleistet werden, da somit ein Spektrum mit einer Referenz (z. B. gleiche Speiseölsorte) verglichen werden kann, unabhängig davon, in welchem Labor, an welchem Instrument, in welchem Jahr eine Probe analysiert wurde und somit eine Datenbank aufgebaut werden kann, die valide und vergleichbare Ergebnisse liefert sowie gerichtsfest eingesetzt werden kann.

6 Schlussfolgerungen und Ausblick

Um *Food Fraud* frühzeitig zu entdecken, wächst der Bedarf an nicht-zielgerichteten Analyseverfahren. Es ist jedoch zurzeit noch schwierig diese Techniken in der Routine einzusetzen, da verschiedene Herausforderungen im Bereich der Harmonisierung/Standardisierung überwunden werden müssen. In der vorliegenden Dissertation wurden daher Harmonisierungs-/Standardisierungsaspekte für die Anwendung von nicht-zielgerichteten Analyseverfahren für die Routine u. a. in der amtlichen Lebensmittelüberwachung diskutiert und Lösungsansätze vorgestellt.

Im Rahmen dieser Arbeit wurden Analyseverfahren auf ihre Eignung untersucht, welche auf der FT-MIR-, FT-Raman und ^{1}H-NMR-Spektroskopie in Kombination mit einer multivariaten Regressionsanalyse (PLS-R) beruhen und im Vergleich zu den üblicherweise eingesetzten chromatographischen Verfahren zeitsparender und mit weniger Laboraufwand verbunden sind. Eine Literaturrecherche zeigte, dass sich Ansätze in bereits publizierten Studien zur Quantifizierung von Verfälschungen in Speiseölen in Bezug auf die Anzahl der Proben für die Modellberechnung und -entwicklung, auf die verwendeten Validierungsstrategien, auf die Berechnungsgrundlage für die MDL/LOD sowie die *performance* Parameter voneinander unterscheiden. Die Vielzahl an genutzten Herangehensweisen und Bewertungsgrundlagen für derartige Modelle demonstriert, dass Laboratorien üblicherweise eine individuelle Vorgehensweise entwickeln und verfolgen. Um spektroskopie-basierte Verfahren in Kombination mit der multivariaten Datenanalyse zu harmonisieren, sollte daher in Zukunft der Schwerpunkt auf der Entwicklung eines einheitlichen Ansatzes für die Modellentwicklung und -validierung, Methodenvalidierung, die Bewertungsstrategien und die Berechnung der Mindestnachweisgrenzen liegen, um die mit verschiedenen spektroskopischen

Techniken erzielten Ergebnisse besser vergleichen zu können. Dazu gehört u. a. die Anwendung einer einheitlichen Validierungsstrategie wie sie von Riedl *et al.* vorgeschlagen wurde (Riedl et al., 2015). Nur so kann es möglich werden diese Verfahren auch routinemäßig einzusetzen und die Datenvalidität zu steigern, sodass Ergebnisse ebenfalls in Gerichtsverfahren Bestand hätten. Der Schlüssel hierzu ist zudem eine angemessene Qualitätssicherung. Mit Hilfe von *outlier score*-basierten Modellen wurde in der vorliegenden Arbeit eine multivariate Kontrollkarte in Anlehnung an die zielgerichtete Analytik konzipiert. Diese kann allgemein angewendet und an andere Lebensmittelmatrices und Techniken (z. B. NMR- oder Raman-Spektroskopie) angepasst werden, da die analytische Variation einer Vorperiode der QS-Messungen in die Berechnung einbezogen wird. Bezüglich der Evaluierung der entwickelten multivariaten Kontrollkarte wäre es denkbar, dass andere Labore diese nutzen, an deren spezifische Bedürfnisse anpassen und optimieren (andere Matrices, andere Authentizitätsfragestellungen etc.).

Ein weiterer wesentlicher Schritt zur Harmonisierung der nicht-zielgerichteten Analytik ist die Entwicklung von Ansätzen, die die Vergleichbarkeit von Spektraldaten gewährleisten, die mit verschiedenen Instrumenten (unter Verwendung derselben Technik) gewonnen wurden, um gemeinsame Datenbanken nutzen zu können. In der vorliegenden Dissertation wurden verschiedene Ansätze untersucht. Um die praktische Umsetzbarkeit der diskutierten Ansätze zu überprüfen, sollte eine Pilotstudie unter Einbeziehung mehrerer Hersteller von MIR-Instrumenten und mehrerer Laboratorien durchgeführt werden. Zudem könnten Eignungsprüfungen durchgeführt werden, mit der die sichere Anwendung der Datenbank in den einzelnen Laboren geprüft werden könnte.

In Summe liefert die vorliegende Arbeit wichtige Instrumente und Denkanstöße zur erfolgreichen Gewährleistung der Vergleichbarkeit sowie Optimierung der Qualität nicht-zielgerichteter spektroskopischer Daten. Bei Umsetzung der beschriebenen Ansätze und Vorschläge durch weitere Labore kann von einer Festigung der Stellung der nicht-zielgerichteten, spektroskopie-basierten Authentizitätsprüfung als ein substanzielles Element der Routineanalytik ausgegangen werden.

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8 Abbildungsverzeichnis

9 Tabellenverzeichnis

10 Anhang

10.1 Ergänzende Informationen zur Publikation „*Comparison of spectroscopic techniques using the adulteration of pumpkin seed oil as example*“ in der Fachzeitschrift *Food Analytical Methods*

Table S1 Sample set of edible seed oils with different varieties; pumpkin seed oil n = 80, rapeseed oil n = 44

Variety	**Type of processing**	**Organic agriculture**	**Country of origin**
Pumpkin seed oil	Cold pressed	Yes	EU-/Non-EU-agriculture
Pumpkin seed oil	Cold pressed	Yes	Austria
Pumpkin seed oil	Cold pressed	No	Austria
Pumpkin seed oil	Cold pressed	No	No information
Pumpkin seed oil	Cold pressed	No	Austria
Pumpkin seed oil	Cold pressed	Yes	Austria
Pumpkin seed oil	Cold pressed	Yes	Germany
Pumpkin seed oil	Cold pressed	Yes	Austria
Pumpkin seed oil	Cold pressed	No	Austria
Pumpkin seed oil	Cold pressed	No	No information
Pumpkin seed oil	Virgin	Yes	Germany
Pumpkin seed oil	Cold pressed	Yes	Hungary
Pumpkin seed oil	Cold pressed	Yes	Austria
Pumpkin seed oil	Virgin	Yes	Germany
Pumpkin seed oil	Cold pressed	Yes	Austria
Pumpkin seed oil	Cold pressed	Yes	Austria

Pumpkin seed oil	Cold pressed	No	No information
Pumpkin seed oil	No information	No	No information
Pumpkin seed oil	Cold pressed	No	Austria
Pumpkin seed oil	Cold pressed	Yes	Non-EU-agriculture
Pumpkin seed oil	Cold pressed	Yes	Austria
Pumpkin seed oil	Refined	Yes	EU-agriculture
Pumpkin seed oil	Cold pressed	Yes	Austria
Pumpkin seed oil	Cold pressed	Yes	Austria
Pumpkin seed oil	Cold pressed	Yes	Austria
Pumpkin seed oil	Cold pressed	No	EU-/Non-EU-agriculture
Pumpkin seed oil	Cold pressed	Yes	Austria
Pumpkin seed oil	Cold pressed	Yes	Austria
Pumpkin seed oil	Cold pressed	No	Austria
Pumpkin seed oil	Refined	No	No information
Pumpkin seed oil	Cold pressed	No	Austria
Pumpkin seed oil	Cold pressed	Yes	Austria
Pumpkin seed oil	Cold pressed	Yes	Austria
Pumpkin seed oil	Cold pressed	No	Austria
Pumpkin seed oil	Cold pressed	No	Austria
Pumpkin seed oil	Cold pressed	No	Austria
Pumpkin seed oil	Cold pressed	Yes	Austria
Pumpkin seed oil	Cold pressed	Yes	EU-agriculture
Pumpkin seed oil	Cold pressed	Yes	Austria

Pumpkin seed oil	Virgin	Yes	EU-agriculture
Pumpkin seed oil	Cold pressed	No	Austria
Pumpkin seed oil	Cold pressed	No	Austria
Pumpkin seed oil	Cold pressed	No	Austria
Pumpkin seed oil	No information	Yes	Germany
Pumpkin seed oil	No information	Yes	Germany
Pumpkin seed oil	No information	No	Austria
Pumpkin seed oil	No information	No	Great Britain
Pumpkin seed oil	Cold pressed	Yes	Austria
Pumpkin seed oil	Cold pressed	Yes	Germany
Pumpkin seed oil	No information	No	Austria
Pumpkin seed oil	No information	No	Germany
Pumpkin seed oil	No information	No	Germany
Pumpkin seed oil	No information	Yes	Germany
Pumpkin seed oil	No information	No	Austria
Pumpkin seed oil	Cold pressed	No	Austria
Pumpkin seed oil	No information	No	Austria
Pumpkin seed oil	No information	No	Austria
Pumpkin seed oil	Cold pressed	Yes	Non-EU-agriculture
Pumpkin seed oil	Cold pressed	Yes	Non-EU-agriculture
Pumpkin seed oil	No information	No	Germany
Pumpkin seed oil	No information	No	Germany
Pumpkin seed oil	No information	No	Austria

Pumpkin seed oil	No information	Yes	Austria
Pumpkin seed oil	No information	Yes	Austria
Pumpkin seed oil	No information	Yes	Austria
Pumpkin seed oil	No information	Yes	Germany
Pumpkin seed oil	No information	Yes	Germany
Pumpkin seed oil	Cold pressed	No	Austria
Pumpkin seed oil	No information	No	Austria
Pumpkin seed oil	No information	No	Austria
Pumpkin seed oil	No information	No	Austria
Pumpkin seed oil	No information	No	Austria
Pumpkin seed oil	Cold pressed	Yes	EU-agriculture
Pumpkin seed oil	No information	Yes	Austria
Pumpkin seed oil	No information	No	Great Britain
Pumpkin seed oil	Cold pressed	no	Netherlands
Pumpkin seed oil	No information	Yes	Austria
Pumpkin seed oil	Cold pressed	No	Germany
Pumpkin seed oil	No information	Yes	Austria
Pumpkin seed oil	No information	Yes	No information
Rapeseed oil	Refined	No	Germany
Rapeseed oil	Cold pressed	No	Germany
Rapeseed oil	Refined	No	Germany
Rapeseed oil	Virgin	No	No information
Rapeseed oil	Cold pressed	No	Germany
Rapeseed oil	Virgin	No	Germany
Rapeseed oil	Refined	No	No information

Rapeseed oil	Refined	No	Austria
Rapeseed oil	Refined	No	No information
Rapeseed oil	Virgin	No	No information
Rapeseed oil	Virgin	Yes	EU-agriculture
Rapeseed oil	Cold pressed	Yes	EU-/Non-EU-agriculture
Rapeseed oil	Refined	No	Germany
Rapeseed oil	Virgin	No	Germany
Rapeseed oil	Cold pressed	No	Germany
Rapeseed oil	Cold pressed	No	Germany
Rapeseed oil	Cold pressed	No	Germany
Rapeseed oil	Refined	No	No information
Rapeseed oil	Virgin	No	Germany
Rapeseed oil	Refined	No	Austria
Rapeseed oil	Refined	No	No information
Rapeseed oil	Refined	No	Germany
Rapeseed oil	Cold pressed	No	Germany
Rapeseed oil	Virgin	No	Germany
Rapeseed oil	Refined	No	No information
Rapeseed oil	Refined	No	No information
Rapeseed oil	Refined	No	No information
Rapeseed oil	Virgin	No	Germany
Rapeseed oil	Virgin	Yes	EU-agriculture
Rapeseed oil	Cold pressed	No	Germany
Rapeseed oil	Cold pressed	Yes	EU-agriculture

Rapeseed oil	Refined	No	Germany
Rapeseed oil	Virgin	Yes	EU-agriculture
Rapeseed oil	Virgin	Yes	France
Rapeseed oil	Virgin	Yes	EU-agriculture
Rapeseed oil	Refined	No	No information
Rapeseed oil	Refined	No	No information
Rapeseed oil	Refined	No	No information
Rapeseed oil	Refined	No	No information
Rapeseed oil	Refined	No	No information
Rapeseed oil	Refined	No	No information
Rapeseed oil	Refined	No	No information
Rapeseed oil	Refined	No	No information
Rapeseed oil	Refined	No	No information

Table S2 Adulteration of pool samples 1 and 2 with rapeseed oil samples as adulterant, respectively

Adulteration level [%]

Pool sample 1				**Pool sample 2**			
R 1	**R 2**	**R 3**	**R 4**	**R 1**	**R 2**	**R 3**	**R 4**
0.53	0.51	0.55	0.50	0.51	0.59	0.52	0.52
1.02	1.07	1.05	1.13	0.99	1.09	0.93	0.99
2.66	2.02	2.40	1.96	2.86	2.52	2.22	2.02
3.02	3.04	3.11	2.99	2.99	2.94	3.06	2.99
4.01	4.10	4.32	3.98	4.14	4.12	4.22	3.82
4.95	5.01	5.02	4.99	5.06	5.00	5.01	5.00

6.20	6.28	6.00	6.08	5.96	6.20	6.46	6.06
7.15	6.98	7.11	7.02	6.99	7.12	6.97	7.05
8.34	8.30	8.84	8.26	7.82	8.08	7.80	8.32
9.18	8.68	9.18	9.38	9.66	8.80	9.06	9.16
9.91	10.01	10.30	9.87	9.86	9.90	10.06	10.06
19.77	20.22	20.27	20.42	20.05	19.82	19.88	20.33
30.71	30.17	30.33	29.87	29.78	29.89	29.88	30.24
39.98	39.98	40.43	40.13	39.90	39.76	40.11	40.31
49.92	49.93	49.88	50.18	49.89	49.93	49.84	50.17
60.24	59.82	60.47	59.82	59.86	60.31	60.09	59.97
70.38	70.02	69.95	70.12	70.17	69.77	70.11	69.91
79.95	80.00	79.98	79.83	79.83	79.85	80.02	80.20
89.80	90.01	90.40	90.00	90.01	89.91	89.94	90.59

R = rapeseed oil.

Table S3 Adulteration of pool samples 3 and 4 with rapeseed oil samples as adulterant, respectively

Adulteration level [%]

Pool sample 3				**Pool sample 3**			
R 1	**R 2**	**R 3**	**R 4**	**R 1**	**R 2**	**R 3**	**R 4**
0.911	0.994	1.003	1.076	0.975	0.892	0.948	0.920
1.840	1.941	1.803	1.868	1.831	1.849	1.849	1.831
5.520	5.492	5.695	5.465	5.492	5.548	5.548	5.456
9.182	9.136	9.154	9.218	9.476	9.126	9.145	9.228
12.963	12.834	12.908	13.000	12.871	9.182	12.908	12.889

R = rapeseed oil.

Table S4 Used chemicals for ^{1}H-NMR spectroscopy

Chemical	**Producer**
Deuterium chloroform ($CDCl_3$, 99.8 %) with 0.03 % Tetramethylsilan (TMS) as internal standard	Merck KGaA, Darmstadt, Germany
Deuterium methanol, ≥ 99,8 %	Merck KGaA, Darmstadt, Germany
Sucrose solution	Bruker Corporation, Billerica Germany
Extran®MA 03, phosphatefree	Merck KGaA, Darmstadt, Germany
Acetone, p.a., ≥ 99,9 %	Merck KGaA, Darmstadt, Germany

Table S1 ^{1}H-NMR instrument specifications

	Instrument specifications
spectrometer type	Ascend™ 400
probe	5 mm PASEI 1H/D-13C Z-GRD
NMR console	Avance III
autosampler	BACS-120
magnetic field strength	400.27 MHz
temperature control	BCU I -40/50
software versions	TopSpin 3.5pl2
	IconNMR 5.0.2

Table S2 Specific parameters for ^{1}H-NMR single experiment

Pulse program	TD	NS	DS	D1 (s)	SW (ppm)	RG	AQ (s)	Temperature
zg	65536	16	4	4	20.545	4	3.9845889	300.0 K ± 0.1 K

TD = time domain size; NS = scans to execute; DS = number of dummy scans; D1 (s) = relaxation delay in s; SW (ppm) = spectral width in ppm; RG = receiver gain; AQ (s) = acquisition time in s.

Table S3 Quality assuring parameters of the two reference solutions

Reference solution	Parameters	Assessment limits
Deuterated methanol (methanol-d_4)	Absolute temperature of methanol during measurement	300.1 K Δ_{max}: ± 0.2 K
Sucrose solution	Water suppression	
	• Length of the 90° pulse	• 10.0 µs Δmax: ± 0.3 µs
	• Half-width of the Tetramethylsilan (TMS) signal	• ~ 0.5 Hz, maximum 0.7 Hz
	• Signal to noise ratio	• maximum 179

Table S4 Applied pre-processing combinations for improvement of PLS-R results for MIR spectral data

PP	1st step	2nd step	3rd step	4th step
1	SNV	1st derivative, SG	Smooth, SG	Mean center
2	SNV	1st derivative, SG	Smooth, SG	Pareto scaling
3	MSC (median)	1st derivative, SG	Smooth, SG	Mean center
4	1st derivative, SG	Mean Center		

5	2nd derivative, SG	Mean Center	
6	Normalization	2nd derivative, SG	Mean Center
7	Normalization	1st derivative, SG	Mean Center
8	SNV	Mean Center	
9	SNV	1st derivative, SG	Mean Center

PP = pre-processing; SNV = standard normal variate; SG = Savitzky-Golay polynomial derivative filter (1st and 2nd order and centred 11 point window); MSC = Multiplicative scatter correction.

Table S5 Applied pre-processing combinations for improvement of PLS-R results for Raman spectral data

PP	1st step	2nd step	3rd step	4th step
1	SNV	1st derivative, SG	Smooth, SG	Mean center
2	SNV	1st derivative, SG	Smooth, SG	Pareto scaling
3	MSC (median)	1st derivative, SG	Smooth, SG	Mean center
4	1st derivative, SG	Mean Center		
5	2nd derivative, SG	Mean Center		
6	Normalization	2nd derivative, SG	Mean Center	
7	Normalization	1st derivative, SG	Mean Center	
8	SNV	Mean Center		
9	SNV	1st derivative, SG	Mean Center	

PP = pre-processing; SNV = standard normal variate; SG = Savitzky-Golay polynomial derivative filter (1st and 2nd order and centred 11 point window); MSC = Multiplicative scatter correction.

Table S6 Applied pre-processing (single and combinations) for improvement of PLS-R results for ^{1}H-NMR spectral data

PP	1st step	2nd step
1	Normalization	Mean center
2	Normalization	Pareto scaling
3	Mean center	
4	Pareto scaling	
5	Variance scaling	

6	Normalization	Variance scaling	

PP = pre-processing; Normalization to α-signals of glycerol 3.9 ppm - 4.56 ppm.

Table S7 PLS-R results of internal and external validation for various pre-processing steps for each method

		Parameter						
PP	**method**	LV	$R^2_{Cal.}$	R^2_{CV}	$R^2_{Pred.}$	RMSEC	RMSECV	RMSEP
1	MIR	4	0.993	0.986	0.989	2.196	3.122	2.894
	Raman	2	0.976	0.961	0.978	4.370	5.564	4.099
	^{1}H-NMR	2	0.998	0.997	0.957	1.381	1.644	1.485
2	MIR	3	0.988	0.983	0.981	2.971	3.539	3.081
	Raman	3	0.984	0.953	0.975	2.518	4.785	6.232
	^{1}H-NMR	3	0.998	0.998	0.959	1.148	1.326	1.422
3	MIR	4	0.993	0.986	0.989	2.196	3.122	2.895
	Raman	2	0.976	0.949	0.979	4.306	6.249	3.963
	^{1}H-NMR	2	0.996	0.996	0.997	1.626	1.776	2.120
4	MIR	3	0.992	0.982	0.799	4.987	6.308	8.724
	Raman	3	0.990	0.944	0.958	2.633	6.073	5.645
	^{1}H-NMR	2	0.995	0.995	0.997	1.808	1.959	38.654
5	MIR	2	0.991	0.983	0.981	2.611	3.659	3.315
	Raman	2	0.967	0.938	0.960	4.382	7.141	6.177
	^{1}H-NMR	4	0.998	0.995	0.996	1.289	1.811	24.066
6	MIR	4	0.991	0.973	0.998	3.056	4.731	3.315
	Raman	1	0.958	0.949	0.787	6.794	7.365	10.610
	^{1}H-NMR	4	0.998	0.996	0.997	1.198	1.729	38.945
7	MIR	3	0.984	0.971	0.980	3.036	4.222	3.909
	Raman	1	0.956	0.954	0.819	6.862	6.965	10.422
	^{1}H-NMR				-			
8	MIR	3	0.991	0.983	0.993	3.136	4.012	2.566
	Raman	3	0.988	0.962	0.983	3.202	4.465	4.560
	^{1}H-NMR				-			
9	MIR	3	0.994	0.975	0.987	1.939	4.164	2.687
	Raman	2	0.972	0.920	0.976	7.132	10.913	13.211
	^{1}H-NMR				-			

PP = pre-processing; LV = latent variables; $R^2_{Cal.}$ = determination coefficient of calibration; R^2_{CV} = determination coefficient of cross validation; $R^2_{Pred.}$ = determination coefficient of prediction; RMSEC = root mean square error of calibration; RMSECV = root mean square error of cross validation; RMSEP = root mean square error of prediction.

10.2 Ergänzende Informationen zur Publikation „*Quality control of spectroscopic data in non-targeted analysis – development of a multivariate control chart*" in der Fachzeitschrift *Food Control*

Table S1: Sample set of edible oils (n = 150) with different varieties.

Variety	**type of processing**	**organic agriculture**	**country of origin**
black cumin oil	cold pressed	no	Palestine
black cumin oil	cold pressed	no	Palestine
grape seed oil	refined	no	Germany
grape seed oil	refined	no	no information
grape seed oil	refined	no	no information
hazelnut oil	refined	no	no information
hemp seed oil	virgin	yes	Austria
hemp seed oil	cold pressed	yes	EU-/Non-EU-agriculture
linseed oil	cold pressed	no	no information
linseed oil	cold pressed	no	no information
linseed oil	cold pressed	yes	Non-EU-agriculture
linseed oil	refined	yes	Romania
linseed oil	cold pressed	no	Germany
linseed oil	cold pressed	yes	EU-agriculture
linseed oil	cold pressed	no	no information
linseed oil	refined	no	Germany
linseed oil	cold pressed	no	no information

linseed oil	cold pressed	yes	Non-EU-agriculture
linseed oil	virgin	yes	Romania
linseed oil	virgin	yes	Germany
linseed oil	cold pressed	yes	Romania
linseed oil	refined	yes	EU-/Non-EU-agriculture
linseed oil	virgin	yes	EU-agriculture
linseed oil	virgin	yes	EU-agriculture (Poland. Romania)
mustard oil	cold pressed	no	Germany
peanut oil	refined	no	no information
peanut oil	refined	no	no information
peanut oil	refined	no	no information
peanut oil	refined	no	no information
peanut oil	refined	no	no information
peanut oil	virgin	yes	China
poppy seed oil	cold pressed	no	Germany
pumpkin seed oil	cold pressed	yes	EU-/Non-EU-agriculture
pumpkin seed oil	cold pressed	yes	Austria
pumpkin seed oil	refined	no	Austria
pumpkin seed oil	refined	no	no information
pumpkin seed oil	refined	no	Austria
pumpkin seed oil	refined	yes	Austria

pumpkin seed oil	refined	yes	Germany
pumpkin seed oil	refined	yes	Austria
pumpkin seed oil	refined	no	Austria
pumpkin seed oil	refined	no	no information
pumpkin seed oil	virgin	yes	Germany
pumpkin seed oil	refined	yes	Hungary
pumpkin seed oil	refined	yes	Austria
pumpkin seed oil	virgin	yes	Germany
pumpkin seed oil	refined	yes	Austria
pumpkin seed oil	refined	yes	Austria
pumpkin seed oil	refined	no	no information
pumpkin seed oil	refined	no	no information
pumpkin seed oil	refined	no	Austria
pumpkin seed oil	cold pressed	yes	Non-EU-agriculture
pumpkin seed oil	refined	yes	Austria
pumpkin seed oil	refined	yes	EU-agriculture
pumpkin seed oil	refined	yes	Austria
pumpkin seed oil	refined	yes	Austria
pumpkin seed oil	refined	yes	Austria
pumpkin seed oil	refined	no	EU-/Non-EU-agriculture
pumpkin seed oil	refined	yes	Austria
pumpkin seed oil	cold pressed	yes	Austria
pumpkin seed oil	refined	no	Austria

pumpkin seed oil	refined	no	no information
pumpkin seed oil	refined	no	Austria
pumpkin seed oil	refined	yes	Austria
pumpkin seed oil	cold pressed	yes	Austria
pumpkin seed oil	cold pressed	no	Austria
pumpkin seed oil	refined	no	Austria
pumpkin seed oil	refined	no	Austria
pumpkin seed oil	refined	yes	Austria
pumpkin seed oil	refined	yes	EU-agriculture
pumpkin seed oil	cold pressed	yes	Austria
pumpkin seed oil	virgin	yes	EU-agriculture
rapeseed oil	refined	no	Germany
rapeseed oil	cold pressed	no	Germany
rapeseed oil	refined	no	Germany
rapeseed oil	virgin	no	no information
rapeseed oil	cold pressed	no	Germany
rapeseed oil	virgin	no	Germany
rapeseed oil	refined	no	no information
rapeseed oil	refined	no	Austria
rapeseed oil	refined	no	no information
rapeseed oil	virgin	no	no information
rapeseed oil	virgin	yes	EU-agriculture
rapeseed oil	cold pressed	yes	EU-/Non-EU-agriculture
rapeseed oil	refined	no	Germany

rapeseed oil	virgin	no	Germany
rapeseed oil	cold pressed	no	Germany
rapeseed oil	cold pressed	no	Germany
rapeseed oil	cold pressed	no	Germany
rapeseed oil	refined	no	no information
rapeseed oil	virgin	no	Germany
rapeseed oil	refined	no	Austria
rapeseed oil	refined	no	no information
rapeseed oil	refined	no	Germany
rapeseed oil	cold pressed	no	Germany
rapeseed oil	virgin	no	Germany
rapeseed oil	refined	no	no information
rapeseed oil	refined	no	no information
rapeseed oil	refined	no	no information
rapeseed oil	virgin	no	Germany
rapeseed oil	virgin	yes	EU-agriculture
rapeseed oil	cold pressed	no	Germany
rapeseed oil	cold pressed	yes	EU-agriculture
rapeseed oil	refined	no	Germany
rapeseed oil	virgin	yes	EU-agriculture
rapeseed oil	virgin	yes	France
rapeseed oil	virgin	yes	EU-agriculture
rapeseed oil	refined	no	no information
rapeseed oil	refined	no	no information
rapeseed oil	refined	no	no information

rapeseed oil	refined	no	no information
rapeseed oil	refined	no	no information
rapeseed oil	refined	no	no information
rapeseed oil	refined	no	no information
rapeseed oil	refined	no	no information
rapeseed oil	refined	no	no information
sesame seed oil	cold pressed	no	Germany
sesame seed oil	cold pressed	yes	EU-/Non-EU-agriculture
sesame seed oil	refined	no	no information
sesame seed oil	refined	yes	Non-EU-agriculture
sesame seed oil	refined	no	no information
sesame seed oil	refined	no	no information
sesame seed oil	virgin	yes	Non-EU-agriculture
sesame seed oil	refined	no	no information
sesame seed oil	refined	no	no information
sesame seed oil	refined	yes	Burkina Faso
soybean oil	refined	no	no information
soybean oil	refined	no	no information
soybean oil	virgin	yes	EU-agriculture
sunflower oil	cold pressed	no	Germany
sunflower oil	refined	no	no information
sunflower oil	virgin	yes	EU-agriculture

sunflower oil	virgin	yes	EU-agriculture
sunflower oil	refined	no	no information
sunflower oil	refined	no	no information
sunflower oil	refined	no	no information
sunflower oil	refined	no	no information
sunflower oil	cold pressed	no	Germany
sunflower oil	virgin	yes	France
sunflower oil	virgin	yes	France
sunflower oil	refined	no	no information
sunflower oil	virgin	yes	EU-agriculture
sunflower oil	virgin	yes	Germany
sunflower oil	virgin	yes	EU-agriculture
sunflower oil	virgin	yes	EU-agriculture
walnut oil	refined	no	no information
walnut oil	refined	no	no information
walnut oil	refined	no	no information
walnut oil	refined	yes	Moldova
walnut oil	virgin	yes	Moldova

Table S2: Outlier scores for each measurement (mean spectrum of a triplicate) of pre-period (n = 15), generated by different outlier score-based approaches.

sample	outlier score			
number	ED	LOF	*k*-NN	KDE
1	1.509	0.992	2.240	0.102
2	1.304	0.992	2.093	0.113
3	1.402	0.992	2.168	0.107
4	1.014	0.992	1.934	0.124

5	2.106	0.992	2.709	0.070
6	1.887	0.992	2.522	0.083
7	1.735	0.998	2.410	0.090
8	1.759	0.992	2.419	0.090
9	1.382	0.992	2.157	0.108
10	1.847	0.992	2.493	0.084
11	1.600	0.992	2.298	0.098
12	1.558	0.992	2.287	0.098
13	1.144	0.992	2.004	0.119
14	1.573	0.992	2.283	0.099
15	1.579	0.992	2.286	0.099

ED = Euclidian distance; *k*-NN = *k*-nearest neighbour; LOF = local outlier factor; KDE = kernel density estimation.

Table S3: Outlier scores for each measurement (mean spectrum of a triplicate) of pre-period (n = 20), generated by different outlier score-based approaches.

sample	outlier score			
number	ED	LOF	*k*-NN	KDE
1	1.522	0.990	2.361	0.093
2	1.350	0.990	2.239	0.102
3	1.476	0.992	2.325	0.096
4	1.160	0.993	2.128	0.110
5	2.331	0.990	2.954	0.059
6	1.868	1.000	2.608	0.077
7	1.806	0.992	2.558	0.080
8	1.771	0.990	2.530	0.082
9	1.411	0.990	2.289	0.098
10	1.930	0.993	2.647	0.075
11	1.636	0.993	2.431	0.089
12	1.778	0.993	2.526	0.083
13	1.177	0.993	2.145	0.108
14	1.559	0.993	2.385	0.092
15	1.719	0.993	2.480	0.086
16	2.156	0.990	2.817	0.066

17	2.112	0.990	2.798	0.065
18	1.864	0.990	2.601	0.078
19	1.831	0.990	2.575	0.079
20	2.168	0.990	2.828	0.065

ED = Euclidian distance; *k*-NN = *k*-nearest neighbour; LOF = local outlier factor; KDE = kernel density estimation.

Table S4: Outlier scores for each measurement (mean spectrum of a triplicate) of pre-period (n = 25), generated by different outlier score-based approaches.

sample	outlier score			
number	ED	LOF	*k*-NN	KDE
1	1.574	0.992	2.321	0.088
2	1.416	0.986	2.202	0.095
3	1.583	0.995	2.293	0.088
4	1.342	0.994	2.111	0.100
5	2.569	1.061	2.941	0.050
6	1.970	1.000	2.597	0.070
7	1.989	1.005	2.553	0.071
8	1.879	0.984	2.489	0.075
9	1.452	0.984	2.219	0.093
10	2.056	1.004	2.604	0.068
11	1.666	1.000	2.351	0.085
12	1.945	1.011	2.500	0.074
13	1.295	0.996	2.129	0.100
14	1.546	0.991	2.301	0.089
15	1.776	0.996	2.421	0.080
16	1.973	0.995	2.559	0.071
17	1.929	0.991	2.547	0.072
18	1.729	0.995	2.427	0.080
19	1.663	0.992	2.365	0.084
20	2.038	0.999	2.617	0.068
21	1.631	0.992	2.318	0.086
22	2.102	0.999	2.651	0.065
23	2.217	1.013	2.689	0.063

24	1.871	0.994	2.469	0.076
25	2.281	1.015	2.758	0.059

ED = Euclidian distance; *k*-NN = *k*-nearest neighbour; LOF = local outlier factor; KDE = kernel density estimation.

Table S5: Outlier scores for each measurement (mean spectrum of a triplicate) of pre-period (n = 30), generated by different outlier score-based approaches.

sample	outlier score			
number	ED	LOF	*k*-NN	KDE
1	1.626	0.986	2.258	0.086
2	1.451	0.991	2.123	0.095
3	1.672	0.988	2.258	0.085
4	1.467	1.001	2.081	0.095
5	2.685	1.122	2.919	0.046
6	2.005	1.024	2.534	0.069
7	2.071	1.030	2.509	0.068
8	1.973	1.001	2.457	0.072
9	1.501	0.985	2.159	0.092
10	2.145	1.038	2.580	0.065
11	1.690	0.984	2.277	0.084
12	2.073	1.044	2.481	0.069
13	1.360	0.989	2.084	0.098
14	1.587	0.991	2.246	0.088
15	1.865	1.013	2.400	0.077
16	1.866	1.007	2.338	0.078
17	1.680	0.994	2.308	0.084
18	1.573	0.991	2.190	0.090
19	1.943	1.009	2.431	0.074
20	2.040	1.013	2.495	0.070
21	2.137	1.011	2.501	0.067
22	1.814	0.997	2.312	0.080
23	2.186	1.024	2.561	0.064

24	1.702	0.992	2.235	0.085
25	1.997	1.000	2.461	0.071
26	1.693	0.994	2.229	0.086
27	1.696	0.992	2.242	0.085
28	1.814	0.984	2.389	0.078
29	1.485	0.985	2.180	0.093
30	1.388	0.989	2.076	0.098

ED = Euclidian distance; *k*-NN = *k*-nearest neighbour; LOF = local outlier factor; KDE = kernel density estimation.

Table S6: Calculated outlier scores for each measurement (mean spectrum of a triplicate) from individual variation based on mean value of pre-period (n = 15), generated by different outlier score-based approaches.

sample name	outlier score			
	ED	LOF	*k*-NN	KDE
$V_{standard}$	2.572	1.034	3.008	0.053
	2.417	1.015	2.868	0.062
	2.270	1.006	2.757	0.067
	2.224	1.005	2.715	0.070
	2.569	1.039	3.003	0.054
	2.088	1.007	2.583	0.080
	2.661	1.045	3.078	0.050
	2.798	1.082	3.188	0.046
	2.494	1.026	2.937	0.057
	2.851	1.090	3.242	0.043
	2.359	1.021	2.819	0.064
	2.600	1.046	3.022	0.054

2.339	1.018	2.795	0.066
2.307	1.017	2.769	0.068
2.285	1.011	2.762	0.067
2.047	0.998	2.565	0.080
2.054	0.998	2.572	0.079
1.885	0.992	2.442	0.088
2.477	1.024	2.910	0.060
2.136	0.999	2.639	0.075
2.064	0.994	2.584	0.078
2.193	1.001	2.685	0.072
2.035	0.992	2.563	0.079
2.668	1.067	3.095	0.049
1.820	0.992	2.389	0.091
1.547	0.992	2.197	0.104
2.213	1.008	2.696	0.072
1.933	0.994	2.476	0.085
1.701	0.992	2.309	0.096
1.831	0.992	2.410	0.089
1.865	0.992	2.422	0.089
1.630	0.992	2.254	0.101
1.949	0.995	2.492	0.084
1.788	0.992	2.367	0.093
1.761	0.992	2.334	0.096
2.158	0.999	2.657	0.074

	1.973	0.995	2.509	0.083
	2.380	1.020	2.841	0.063
	2.792	1.100	3.185	0.046
	2.436	1.026	2.888	0.060
	2.089	0.994	2.611	0.076
	1.959	0.992	2.510	0.082
	1.841	0.992	2.416	0.089
	1.480	0.992	2.149	0.108
	1.571	0.992	2.202	0.105
	1.845	0.994	2.413	0.089
	1.874	0.992	2.434	0.088
$V_{operator}$	2.033	0.992	2.566	0.079
	2.213	1.001	2.715	0.069
	1.901	0.992	2.466	0.085
	2.218	1.000	2.712	0.070
	2.489	1.034	2.926	0.059
	3.750	1.380	4.068	0.016
	4.184	1.513	4.470	0.009
	4.686	1.675	4.943	0.004
	4.859	1.733	5.108	0.003
$VT_{20°C}$	4.131	1.498	4.420	0.010
	4.079	1.484	4.373	0.010
	4.005	1.455	4.303	0.011
	4.149	1.504	4.437	0.009

	2.964	1.123	3.345	0.038
	2.785	1.077	3.193	0.044
	2.906	1.109	3.299	0.040
	2.870	1.107	3.266	0.041
$VT_{25°C}$	2.868	1.100	3.266	0.041
	2.438	1.020	2.889	0.060
	2.590	1.039	3.022	0.053
	2.801	1.082	3.201	0.045
	2.806	1.085	3.207	0.044
	3.592	1.316	3.914	0.020
	3.517	1.298	3.847	0.021
	3.381	1.252	3.719	0.025
	3.466	1.273	3.795	0.023
	3.420	1.264	3.755	0.024
$VT_{35°C}$	3.331	1.239	3.676	0.026
	3.196	1.197	3.545	0.032
	3.190	1.187	3.542	0.032
	3.542	1.298	3.863	0.021
	3.363	1.251	3.706	0.025
	4.592	1.641	4.852	0.005
	4.792	1.707	5.041	0.004
$VT_{40°C}$	4.758	1.692	5.009	0.004
	5.395	1.890	5.612	0.002
	4.594	1.640	4.854	0.005

	4.759	1.693	5.010	0.004
	4.856	1.730	5.102	0.003
	4.802	1.708	5.051	0.004
	4.769	1.696	5.018	0.004
	4.690	1.677	4.946	0.004
	1.985	0.994	2.521	0.082
	2.006	0.996	2.543	0.080
	2.043	0.994	2.567	0.079
	2.249	1.005	2.729	0.069
	2.437	1.034	2.876	0.062
VS_{7d}	2.268	1.008	2.746	0.068
	2.134	0.999	2.633	0.076
	2.112	0.998	2.627	0.075
	2.086	0.997	2.604	0.077
	2.082	1.002	2.585	0.079
	2.648	1.040	3.075	0.050
	2.537	1.025	2.984	0.054
	2.554	1.034	2.990	0.054
	2.374	1.016	2.841	0.062
VS_{14d}	2.125	0.993	2.642	0.074
	1.910	0.992	2.469	0.085
	2.143	0.997	2.654	0.073
	2.200	0.996	2.695	0.071
	1.904	0.992	2.467	0.085

	2.761	1.073	3.170	0.046
	2.409	1.012	2.864	0.061
	2.584	1.032	3.017	0.053
	2.650	1.059	3.077	0.050
	2.441	1.024	2.899	0.059
VS_{21d}	2.694	1.057	3.113	0.048
	2.685	1.054	3.109	0.048
	2.648	1.059	3.075	0.050
	2.691	1.063	3.115	0.048
	2.556	1.037	3.001	0.053
	2.800	1.084	3.206	0.044
	2.542	1.031	2.979	0.055
	2.481	1.009	2.932	0.057
	2.298	0.996	2.781	0.065
	2.495	1.023	2.941	0.057
VS_{28d}	2.301	0.998	2.786	0.065
	2.476	1.024	2.935	0.056
	2.636	1.051	3.069	0.050
	2.471	1.016	2.927	0.057
	2.904	1.114	3.298	0.040
	8.000	2.772	8.154	4.61E-06
	7.701	2.672	7.860	1.02E-05
$VV_{pumpkin}$	7.588	2.636	7.751	1.32E-05
	7.519	2.616	7.684	1.53E-05

	8.272	2.866	8.421	2.26E-06
	8.261	2.862	8.410	2.39E-06
	9.361	3.231	9.493	9.86E-08
	7.865	2.732	8.022	6.55E-06
	7.809	2.711	7.967	7.52E-06
	7.781	2.700	7.939	8.32E-06
	3.489	1.290	3.821	0.022
	2.585	1.034	3.027	0.052
	2.961	1.132	3.350	0.037
	3.215	1.215	3.574	0.029
	3.016	1.155	3.404	0.035
$VV_{rapeseed}$	2.958	1.136	3.352	0.037
	2.690	1.066	3.115	0.047
	4.647	1.653	4.899	0.005
	3.485	1.283	3.809	0.023
	3.188	1.207	3.535	0.032
	8.656	2.994	8.799	7.78E-07
	9.127	3.153	9.263	1.99E-07
	10.686	3.679	10.802	1.34E-09
	8.799	3.042	8.940	5.23E-07
$VV_{sunflower}$	8.669	2.999	8.812	7.46E-07
	8.479	2.936	8.624	1.28E-06
	9.145	3.158	9.280	1.91E-07
	10.908	3.751	11.021	6.04E-10

	10.624	3.658	10.741	1.62E-09
	9.881	3.407	10.006	1.97E-08
	8.394	2.901	8.540	1.70E-06
	8.270	2.860	8.419	2.36E-06
	7.901	2.735	8.057	6.10E-06
	8.385	2.898	8.532	1.72E-06
	8.194	2.834	8.344	2.86E-06
$V_{instrument}$	8.316	2.874	8.464	2.06E-06
	8.194	2.832	8.344	2.89E-06
	7.837	2.713	7.994	7.29E-06
	8.383	2.900	8.530	1.74E-06
	8.122	2.810	8.274	3.41E-06

Variation of $V_{standard}$ = Measurement over a time period of 36 months using standard parameters; $V_{operator}$ = operator using standard parameters; VT = temperature; VS = storage (26.0°C ± 0.5°C); $VV_{rapeseed}$ = variety rapeseed oil; $VV_{pumpkin}$ = variety pumpkin seed oil, $VV_{sunflower}$ = variety sunflower oil; $V_{instrument}$ = measurements on second, identically constructed FT-MIR instrument; CI = confidence interval; ED = Euclidian distance; *k*-NN = *k*-nearest neighbour; LOF = local outlier factor; KDE = kernel density estimation.

Table S7: Calculated outlier scores for each measurement (mean spectrum of a triplicate) from individual variation based on mean value of pre-period (n = 20), generated by different outlier score-based approaches.

sample name	outlier score			
	ED	*k*-NN	LOF	KDE
	1.859	2.527	0.990	0.083
$V_{standard}$	2.362	2.917	1.001	0.060

2.482	3.001	1.019	0.056
2.149	2.742	0.990	0.070
2.527	3.043	1.024	0.054
2.067	2.685	0.990	0.073
2.341	2.901	1.001	0.060
2.045	2.663	0.990	0.074
2.020	2.647	0.990	0.075
2.029	2.665	0.990	0.073
1.780	2.480	0.990	0.085
1.697	2.407	0.990	0.091
1.655	2.396	0.990	0.091
2.349	2.908	0.991	0.060
1.838	2.519	0.990	0.083
1.793	2.494	0.990	0.084
1.915	2.576	0.990	0.079
1.735	2.448	0.990	0.087
2.363	2.920	1.007	0.059
1.557	2.327	0.990	0.095
1.591	2.345	0.990	0.095
2.040	2.673	0.990	0.073
1.775	2.480	0.990	0.085
1.579	2.349	0.990	0.093
1.728	2.453	0.990	0.086
1.841	2.522	1.000	0.083

	1.500	2.296	0.990	0.097
	1.836	2.526	0.990	0.082
	1.661	2.401	0.990	0.090
	1.617	2.362	0.990	0.093
	2.009	2.651	0.990	0.074
	1.818	2.512	0.990	0.083
	2.123	2.734	0.992	0.069
	2.552	3.074	1.031	0.051
	2.284	2.867	1.006	0.061
	1.841	2.533	0.990	0.081
	1.794	2.505	0.990	0.082
	1.808	2.509	1.000	0.083
	1.408	2.233	0.990	0.102
	1.455	2.258	0.990	0.100
	1.798	2.497	1.000	0.084
	1.847	2.530	0.993	0.082
$V_{operator}$	2.034	2.673	0.993	0.072
	2.332	2.899	0.994	0.060
	1.768	2.487	0.990	0.084
	2.038	2.680	0.990	0.072
	2.343	2.911	0.996	0.059
	3.916	4.276	1.299	0.013
$VT_{20°C}$	4.377	4.697	1.426	0.007
	4.902	5.187	1.573	0.003

	5.062	5.340	1.619	0.003
	4.295	4.624	1.403	0.008
	4.325	4.642	1.406	0.008
	4.191	4.526	1.374	0.009
	4.373	4.689	1.422	0.008
	2.980	3.444	1.064	0.035
	2.724	3.233	1.025	0.042
	2.870	3.355	1.041	0.037
	2.895	3.373	1.048	0.037
$VT_{25°C}$	2.928	3.399	1.055	0.036
	2.424	2.977	0.998	0.055
	2.556	3.089	1.002	0.049
	2.813	3.300	1.029	0.040
	2.806	3.295	1.031	0.040
	3.322	3.737	1.166	0.026
	3.198	3.620	1.154	0.030
	3.130	3.569	1.123	0.031
	3.162	3.588	1.137	0.031
	3.155	3.589	1.131	0.030
$VT_{35°C}$	3.061	3.509	1.115	0.033
	2.972	3.430	1.093	0.036
	2.927	3.388	1.085	0.038
	3.264	3.681	1.154	0.028
	3.071	3.515	1.122	0.033

	4.397	4.725	1.438	0.007
	4.603	4.917	1.498	0.005
	4.581	4.898	1.489	0.005
	5.282	5.557	1.688	0.002
	4.425	4.753	1.446	0.006
$VT_{40°C}$	4.589	4.905	1.493	0.005
	4.744	5.054	1.542	0.004
	4.577	4.889	1.487	0.005
	4.619	4.934	1.502	0.005
	4.532	4.854	1.480	0.005
	1.687	2.414	0.990	0.090
	1.778	2.490	0.990	0.084
	1.864	2.549	0.990	0.080
	2.034	2.669	0.991	0.073
	2.292	2.865	1.003	0.062
VS_{7d}	2.040	2.675	0.990	0.072
	1.886	2.555	0.990	0.080
	1.900	2.577	0.990	0.078
	1.855	2.542	0.990	0.080
	1.946	2.598	0.990	0.078
	2.364	2.926	0.995	0.058
	2.289	2.875	0.994	0.060
VS_{14d}	2.296	2.872	0.993	0.061
	2.154	2.768	0.990	0.066

	2.046	2.690	0.990	0.071
	1.731	2.458	0.990	0.086
	1.962	2.625	0.990	0.075
	1.980	2.634	0.990	0.075
	1.790	2.502	0.990	0.083
	2.493	3.031	1.015	0.053
	2.238	2.831	0.993	0.063
	2.304	2.877	0.993	0.061
	2.504	3.051	1.020	0.051
	2.277	2.868	0.994	0.060
VS_{21d}	2.411	2.960	1.004	0.057
	2.405	2.961	1.003	0.056
	2.389	2.948	1.009	0.057
	2.457	3.010	1.010	0.053
	2.339	2.918	1.005	0.058
	2.587	3.116	1.017	0.048
	2.339	2.911	0.994	0.059
	2.416	2.979	0.992	0.054
	2.172	2.787	1.000	0.065
VS_{28d}	2.365	2.937	0.998	0.057
	2.203	2.813	0.990	0.063
	2.355	2.936	0.995	0.056
	2.434	2.995	1.004	0.054
	2.288	2.877	0.995	0.060

	2.813	3.309	1.040	0.039
	7.991	8.181	2.501	4.36E-06
	7.724	7.920	2.424	8.91E-06
	7.536	7.736	2.365	1.42E-05
	7.454	7.658	2.344	1.68E-05
	8.199	8.383	2.563	2.74E-06
$VV_{pumpkin}$	8.177	8.362	2.559	2.89E-06
	9.290	9.453	2.890	1.25E-07
	7.820	8.014	2.449	7.06E-06
	7.762	7.957	2.434	7.96E-06
	7.735	7.931	2.428	8.50E-06
	3.656	4.032	1.227	0.018
	2.575	3.111	1.007	0.048
	3.125	3.561	1.097	0.031
	3.341	3.755	1.154	0.025
	2.999	3.473	1.069	0.032
$VV_{rapeseed}$	2.991	3.462	1.067	0.033
	2.821	3.303	1.034	0.041
	4.874	5.153	1.561	0.004
	3.724	4.078	1.244	0.019
	3.384	3.773	1.171	0.026
	8.637	8.812	2.693	7.80E-07
$VV_{sunflower}$	9.124	9.291	2.841	1.89E-07
	10.684	10.827	3.316	1.23E-09

	8.838	9.009	2.751	4.60E-07
	8.673	8.848	2.704	7.02E-07
	8.480	8.659	2.646	1.20E-06
	9.154	9.320	2.848	1.77E-07
	10.925	11.065	3.383	5.48E-10
	10.623	10.766	3.290	1.58E-09
	9.874	10.027	3.064	1.95E-08
	8.330	8.510	2.599	2.12E-06
	8.199	8.382	2.559	3.04E-06
	7.815	8.007	2.446	8.00E-06
	8.288	8.468	2.587	2.56E-06
	8.124	8.308	2.537	3.60E-06
$V_{instrument}$	8.231	8.413	2.571	2.76E-06
	8.108	8.293	2.533	3.82E-06
	7.762	7.955	2.429	9.01E-06
	8.288	8.469	2.588	2.47E-06
	8.012	8.199	2.507	5.04E-06

Variation of $V_{standard}$ = Measurement over a time period of 36 months using standard parameters; $V_{operator}$ = operator using standard parameters; VT = temperature; VS = storage (26.0°C ± 0.5°C); $VV_{rapeseed}$ = variety rapeseed oil; $VV_{pumpkin}$ = variety pumpkin seed oil, $VV_{sunflower}$ = variety sunflower oil; $V_{instrument}$ = measurements on second, identically constructed FT-MIR instrument; CI = confidence interval; ED = Euclidian distance; *k*-NN = *k*-nearest neighbour; LOF = local outlier factor; KDE = kernel density estimation.

Table S8: Calculated outlier scores for each measurement (mean spectrum of a triplicate) from individual variation based on mean value of pre-period (n = 25), generated by different outlier score-based approaches.

sample name	outlier score			
	ED	*k*-NN	LOF	KDE
$V_{standard}$	1.861	2.426	0.992	0.079
	2.154	2.634	0.993	0.066
	1.855	2.412	0.992	0.080
	1.847	2.414	0.991	0.080
	1.914	2.518	1.002	0.075
	1.624	2.307	0.992	0.088
	1.497	2.176	0.992	0.095
	1.600	2.299	0.982	0.089
	2.193	2.687	0.997	0.064
	1.708	2.365	0.992	0.084
	1.681	2.369	0.996	0.085
	1.786	2.409	0.996	0.081
	1.674	2.353	0.993	0.085
	2.321	2.839	1.026	0.057
	1.415	2.183	0.990	0.097
	1.679	2.312	0.998	0.087
	1.943	2.542	0.991	0.073
	1.729	2.391	0.986	0.083
	1.583	2.316	0.987	0.089
	1.664	2.377	0.989	0.085

	1.840	2.466	0.987	0.079
	1.456	2.242	0.984	0.094
	1.842	2.465	0.988	0.078
	1.622	2.332	0.990	0.088
	1.500	2.223	0.993	0.094
	1.882	2.497	0.996	0.077
	1.733	2.396	0.993	0.082
	1.950	2.517	0.994	0.074
	2.350	2.788	1.026	0.058
	2.200	2.741	0.999	0.061
	1.681	2.378	0.995	0.084
	1.785	2.466	0.995	0.079
	1.852	2.497	1.007	0.077
	1.443	2.190	0.986	0.096
	1.401	2.183	0.991	0.098
	1.805	2.452	0.998	0.080
	1.826	2.478	0.990	0.079
$V_{operator}$	2.024	2.622	0.996	0.069
	2.396	2.860	1.028	0.055
	1.795	2.466	0.983	0.079
	1.947	2.586	0.993	0.072
	2.240	2.761	0.995	0.060
	4.020	4.244	1.412	0.011
$VT_{20°C}$	4.453	4.628	1.541	0.006

	4.979	5.084	1.692	0.003
	5.143	5.242	1.744	0.002
	4.359	4.551	1.514	0.007
	4.472	4.604	1.533	0.007
	4.281	4.470	1.488	0.008
	4.504	4.652	1.548	0.006
	2.958	3.351	1.123	0.033
	2.710	3.185	1.072	0.041
	2.853	3.279	1.095	0.036
	2.897	3.322	1.114	0.035
$VT_{25°C}$	2.944	3.346	1.123	0.034
	2.392	2.908	1.014	0.054
	2.533	3.015	1.028	0.048
	2.806	3.227	1.081	0.038
	2.796	3.227	1.083	0.039
	3.117	3.430	1.165	0.031
	3.008	3.328	1.141	0.035
	2.958	3.305	1.125	0.035
	2.949	3.257	1.122	0.037
$VT_{35°C}$	2.985	3.320	1.126	0.034
	2.872	3.223	1.107	0.038
	2.825	3.185	1.083	0.039
	2.763	3.121	1.071	0.042
	3.073	3.366	1.143	0.032

	2.896	3.248	1.114	0.037
	4.342	4.608	1.539	0.007
	4.556	4.799	1.603	0.005
	4.511	4.762	1.591	0.005
	5.189	5.358	1.791	0.002
	4.387	4.658	1.556	0.006
$VT_{40°C}$	4.529	4.777	1.596	0.005
	4.719	4.986	1.666	0.004
	4.501	4.730	1.580	0.006
	4.573	4.822	1.611	0.005
	4.501	4.773	1.595	0.005
	1.528	2.246	0.989	0.092
	1.670	2.381	0.985	0.084
	1.753	2.428	0.986	0.081
	1.870	2.468	0.992	0.077
	2.174	2.694	0.998	0.064
VS_{7d}	1.857	2.454	0.989	0.078
	1.682	2.322	0.991	0.086
	1.790	2.466	0.992	0.079
	1.730	2.414	0.989	0.082
	1.761	2.375	0.994	0.083
	2.206	2.725	1.002	0.062
VS_{14d}	2.115	2.679	0.998	0.065
	2.085	2.616	0.999	0.068

	1.986	2.579	0.994	0.071
	2.001	2.632	0.990	0.069
	1.636	2.371	0.990	0.086
	1.875	2.519	0.993	0.075
	1.834	2.472	0.989	0.078
	1.740	2.448	0.989	0.081
	2.297	2.772	1.015	0.059
	2.141	2.693	0.998	0.064
	2.115	2.640	0.997	0.067
	2.368	2.889	1.020	0.054
	2.142	2.720	1.003	0.064
VS_{21d}	2.236	2.731	1.006	0.062
	2.250	2.769	1.009	0.060
	2.206	2.719	1.011	0.062
	2.324	2.844	1.015	0.056
	2.235	2.807	0.996	0.059
	2.404	2.889	1.023	0.054
	2.215	2.742	0.994	0.061
	2.350	2.890	1.006	0.054
	2.102	2.709	0.996	0.065
VS_{28d}	2.303	2.836	1.006	0.057
	2.131	2.732	0.991	0.063
	2.315	2.895	1.011	0.055
	2.329	2.874	1.016	0.056

	2.195	2.760	1.000	0.061
	2.728	3.194	1.077	0.040
	8.001	8.156	2.712	4.05E-06
	7.706	7.859	2.615	8.92E-06
	7.529	7.692	2.558	1.36E-05
	7.427	7.606	2.539	1.70E-05
	8.183	8.326	2.768	2.66E-06
$VV_{pumpkin}$	8.140	8.279	2.760	2.96E-06
	9.281	9.396	3.125	1.19E-07
	7.811	7.964	2.657	6.81E-06
	7.746	7.912	2.639	7.81E-06
	7.713	7.872	2.628	8.46E-06
	3.791	4.003	1.339	1.57E-02
	2.647	3.100	1.057	4.36E-02
	3.229	3.525	1.183	2.77E-02
	3.490	3.741	1.261	2.14E-02
	3.058	3.468	1.162	2.96E-02
$VV_{rapeseed}$	3.080	3.453	1.159	2.97E-02
	2.955	3.275	1.116	3.58E-02
	4.997	5.095	1.695	3.40E-03
	3.858	4.027	1.352	1.59E-02
	3.555	3.745	1.275	2.25E-02
	8.640	8.769	2.914	7.36E-07
$VV_{sunflower}$	9.124	9.251	3.080	1.81E-07

	10.707	10.807	3.596	1.13E-09
	8.869	8.970	2.983	4.13E-07
	8.679	8.811	2.928	6.59E-07
	8.476	8.620	2.866	1.15E-06
	9.148	9.271	3.083	1.71E-07
	10.932	11.021	3.662	5.12E-10
	10.631	10.715	3.560	1.48E-09
	9.882	9.977	3.315	1.82E-08
	8.366	8.433	2.802	2.07E-06
	8.235	8.305	2.760	2.94E-06
	7.829	7.927	2.634	7.99E-06
	8.308	8.386	2.787	2.53E-06
	8.157	8.228	2.734	3.61E-06
$V_{instrument}$	8.254	8.330	2.768	2.81E-06
	8.130	8.204	2.726	3.95E-06
	7.786	7.870	2.615	9.05E-06
	8.317	8.402	2.792	2.38E-06
	8.024	8.118	2.697	5.25E-06

Variation of $V_{standard}$ = Measurement over a time period of 36 months using standard parameters; $V_{operator}$ = operator using standard parameters; VT = temperature; VS = storage (26.0°C ± 0.5°C); $VV_{rapeseed}$ = variety rapeseed oil; $VV_{pumpkin}$ = variety pumpkin seed oil, $VV_{sunflower}$ = variety sunflower oil; $V_{instrument}$ = measurements on second, identically constructed FT-MIR instrument; CI = confidence interval; ED = Euclidian distance; *k*-NN = *k*-nearest neighbour; LOF = local outlier factor; KDE = kernel density estimation.

Table S9: Calculated outlier scores for each measurement (mean spectrum of a triplicate) from individual variation based on mean value of pre-period (n = 30), generated by different outlier score-based approaches.

sample name	outlier score			
	ED	*k*-NN	LOF	KDE
$V_{standard}$	2.641	2.753	1.082	0.052
	1.595	2.209	0.983	0.090
	2.160	2.586	1.008	0.066
	1.603	2.213	0.987	0.090
	1.643	2.276	0.989	0.088
	1.746	2.313	0.988	0.084
	1.642	2.252	0.989	0.088
	2.321	2.757	1.045	0.058
	1.315	2.029	0.982	0.103
	1.728	2.271	1.001	0.085
	1.909	2.452	0.990	0.075
	1.738	2.340	0.983	0.083
	1.612	2.274	0.981	0.088
	1.664	2.320	0.987	0.086
	1.901	2.445	1.001	0.076
	1.466	2.194	0.984	0.095
	1.847	2.409	0.993	0.078
	1.664	2.313	0.990	0.086
	1.490	2.171	0.977	0.095
	1.907	2.453	0.990	0.076

	1.774	2.382	0.984	0.081
	1.901	2.421	1.004	0.076
	2.304	2.666	1.027	0.060
	2.222	2.710	1.033	0.061
	1.673	2.326	0.986	0.085
	1.824	2.430	0.994	0.078
	2.705	2.979	1.125	0.044
	1.898	2.448	1.006	0.076
	1.460	2.135	0.978	0.096
	1.390	2.108	0.976	0.099
	1.840	2.420	1.006	0.078
	1.871	2.449	1.002	0.077
$V_{operator}$	2.065	2.602	1.014	0.068
	2.476	2.836	1.076	0.052
	1.843	2.432	0.983	0.078
	1.942	2.531	0.994	0.073
	2.208	2.675	1.019	0.062
	4.108	4.243	1.539	0.010
	4.526	4.619	1.674	0.006
	5.054	5.069	1.838	0.003
$VT_{20°C}$	5.214	5.228	1.895	0.002
	4.422	4.540	1.646	0.007
	4.570	4.582	1.662	0.006
	4.364	4.470	1.621	0.007

	4.598	4.634	1.681	0.006
	2.984	3.314	1.210	0.033
	2.739	3.134	1.146	0.040
	2.892	3.265	1.194	0.035
	2.938	3.281	1.198	0.034
$VT_{25°C}$	3.011	3.330	1.216	0.032
	2.425	2.868	1.071	0.053
	2.574	2.996	1.106	0.047
	2.840	3.199	1.168	0.037
	2.838	3.199	1.172	0.037
	3.024	3.209	1.184	0.034
	2.892	3.074	1.141	0.039
	2.884	3.136	1.159	0.038
	2.862	3.053	1.137	0.040
	2.884	3.111	1.149	0.038
$VT_{35°C}$	2.762	3.003	1.123	0.043
	2.767	3.054	1.122	0.041
	2.702	2.985	1.103	0.044
	2.988	3.180	1.176	0.035
	2.800	3.043	1.130	0.041
	4.276	4.481	1.643	0.007
	4.501	4.674	1.713	0.006
$VT_{40°C}$	4.443	4.635	1.698	0.006
	5.137	5.234	1.925	0.002

	4.325	4.541	1.667	0.007
	4.483	4.669	1.711	0.006
	4.683	4.890	1.793	0.004
	4.418	4.569	1.676	0.006
	4.529	4.721	1.729	0.005
	4.472	4.683	1.716	0.005
	1.459	2.142	0.991	0.096
	1.633	2.320	0.993	0.087
	1.745	2.369	0.991	0.082
	1.863	2.413	0.996	0.078
	2.163	2.637	1.022	0.064
VS_{7d}	1.820	2.379	0.998	0.080
	1.653	2.256	0.989	0.088
	1.781	2.410	0.994	0.080
	1.674	2.313	0.988	0.086
	1.749	2.317	0.991	0.083
	2.122	2.573	1.021	0.067
	2.069	2.576	1.012	0.068
	2.049	2.522	1.007	0.070
	1.945	2.491	0.997	0.073
VS_{14d}	2.051	2.621	1.009	0.068
	1.658	2.342	0.977	0.086
	1.896	2.456	0.994	0.075
	1.840	2.414	0.986	0.078

	1.827	2.450	0.998	0.078
	2.281	2.691	1.045	0.060
	2.156	2.638	1.011	0.064
	2.093	2.564	1.014	0.068
	2.365	2.855	1.062	0.054
	2.164	2.698	1.016	0.063
VS_{21d}	2.174	2.595	1.023	0.065
	2.188	2.636	1.028	0.063
	2.176	2.631	1.026	0.064
	2.304	2.766	1.039	0.058
	2.224	2.739	1.033	0.060
	2.399	2.831	1.069	0.054
	2.205	2.684	1.014	0.062
	2.414	2.896	1.068	0.052
	2.142	2.691	1.026	0.064
	2.350	2.818	1.051	0.055
VS_{28d}	2.219	2.744	1.035	0.060
	2.360	2.877	1.064	0.054
	2.360	2.840	1.059	0.055
	2.214	2.723	1.026	0.061
	2.788	3.187	1.168	0.039
	8.026	8.151	2.950	3.84E-06
$VV_{pumpkin}$	7.739	7.854	2.867	8.26E-06
	7.531	7.662	2.783	1.36E-05

	7.436	7.573	2.772	1.70E-05
	8.169	8.274	3.034	2.78E-06
	8.129	8.221	3.013	3.08E-06
	9.259	9.350	3.426	1.25E-07
	7.803	7.920	2.895	7.00E-06
	7.744	7.880	2.885	7.89E-06
	7.705	7.837	2.878	8.64E-06
	3.899	4.009	1.457	0.014
	2.735	3.096	1.135	0.041
	3.351	3.534	1.289	0.025
	3.577	3.731	1.366	0.020
	3.096	3.444	1.253	0.029
$VV_{rapeseed}$	3.143	3.439	1.253	0.028
	3.055	3.277	1.203	0.033
	5.113	5.107	1.852	0.003
	3.991	4.043	1.476	0.014
	3.682	3.748	1.380	0.020
	8.622	8.743	3.174	0.000
	9.112	9.229	3.362	1.82E-07
	10.731	10.794	3.932	1.06E-09
$VV_{sunflower}$	8.871	8.961	3.250	3.93E-07
	8.660	8.779	3.190	6.79E-07
	8.451	8.581	3.118	1.21E-06
	9.127	9.239	3.355	1.76E-07

	10.921	11.002	3.991	5.07E-10
	10.614	10.695	3.874	1.50E-09
	9.856	9.937	3.612	1.89E-08
	8.339	8.366	3.029	2.13E-06
	8.208	8.233	2.988	3.03E-06
	7.790	7.838	2.845	8.58E-06
	8.266	8.291	3.009	2.68E-06
	8.128	8.159	2.962	3.73E-06
$V_{instrument}$	8.225	8.258	2.997	2.91E-06
	8.101	8.132	2.951	4.08E-06
	7.764	7.804	2.832	9.28E-06
	8.296	8.320	3.020	2.44E-06
	7.992	8.034	2.916	5.46E-06

Variation of $V_{standard}$ = Measurement over a time period of 36 months using standard parameters; $V_{operator}$ = operator using standard parameters; VT = temperature; VS = storage (26.0°C ± 0.5°C); $VV_{rapeseed}$ = variety rapeseed oil; $VV_{pumpkin}$ = variety pumpkin seed oil, $VV_{sunflower}$ = variety sunflower oil; $V_{instrument}$ = measurements on second, identically constructed FT-MIR instrument; CI = confidence interval; ED = Euclidian distance; *k*-NN = *k*-nearest neighbour; LOF = local outlier factor; KDE = kernel density estimation.

Table S10: Mean values, standard deviations and warning and action limits based on outlier scores of pre-period QC sample measurements (n = 15, m = 3) for each algorithm.

	ED	***k*-NN**	**LOF**	**KDE**
mean	1.560	2.278	0.993	0.099
SD	0.278	0.204	0.001	0.014
warning limit	2.135	2.696	0.996	0.070

action limit	2.422	2.900	0.997	0.056

SD = standard deviation; ED = Euclidian distance; *k*-NN = *k*-nearest neighbour; LOF = local outlier factor; KDE = kernel density estimation.

Table S11: Mean values, standard deviations and warning and action limits based on outlier scores of pre-period QC sample measurements (n = 20, m = 3) for each algorithm.

	ED	***k*-NN**	**LOF**	**KDE**
mean	1.731	2.511	0.992	0.084
SD	0.324	0.229	0.003	0.015
warning limit	2.380	2.970	0.997	0.055
action limit	2.704	3.200	1.000	0.041

SD = standard deviation; ED = Euclidian distance; *k*-NN = *k*-nearest neighbour; LOF = local outlier factor; KDE = kernel density estimation.

Table S12: Mean values, standard deviations and warning and action limits based on outlier scores of pre-period QC sample measurements (n = 30, m = 3) for each algorithm.

	ED	***k*-NN**	**LOF**	**KDE**
mean	1.806	2.344	1.006	0.080
SD	0.293	0.187	0.028	0.012
warning limit	2.393	2.717	1.061	0.056
action limit	2.686	2.904	1.088	0.044

SD = standard deviation; ED = Euclidian distance; *k*-NN = *k*-nearest neighbour; LOF = local outlier factor; KDE = kernel density estimation.

Table S13: Results of outlier detection for the variations of QC sample, considered separately for CI 95.5 % and CI 99.7 %; calculations based on a defined pre-period (n = 15, m = 3) of QC sample.

generated as	sample name	below CI 95.5 % inlier [%]				CI 95.5 % warning limit* suspect measurements [%]				above CI 99.7 % action limit outlier [%]			
		ED	*k*-NN	LOF	KDE	ED	*k*-NN	LOF	KDE	ED	*k*-NN	LOF	KDE
inlier	$V_{standard}$	45	52	38	55	29	24	0	26	26	24	62	19
	$V_{operator}$	80	70	70	70	20	20	0	30	10	10	30	0
outlier	$VT_{20°C}$	0	0	0	0	0	0	0	0	100	100	100	100
	$VT_{25°C}$	0	0	0	0	0	11	0	11	100	89	100	89
	$VT_{35°C}$	0	0	0	0	0	0	0	0	100	100	100	100
	$VT_{40°C}$	0	0	0	0	0	0	0	0	100	100	100	100
	VS_{7d}	70	70	20	70	20	30	10	30	10	0	70	0
	VS_{14d}	33	56	33	56	33	11	22	11	34	33	45	33
	VS_{21d}	0	0	0	0	10	20	0	20	90	80	100	80
	VS_{28d}	0	0	0	0	20	20	10	60	80	80	90	40
	$VV_{rapeseed}$	0	0	0	0	0	0	0	0	100	100	100	100
	$VV_{pumpkin}$	0	0	0	0	0	0	0	10	100	100	100	90
	$VV_{sunflower}$	0	0	0	0	0	0	0	0	100	100	100	100
	$V_{instrument}$	0	0	0	0	0	0	0	0	100	100	100	100

Variation of $V_{standard}$ = Measurement over a time period of 36 months using standard parameters; $V_{operator}$ = operator using standard parameters; VT = temperature; VS = storage (26.0°C ± 0.5°C); $VV_{rapeseed}$ = variety rapeseed oil; $VV_{pumpkin}$ = variety pumpkin seed oil, $VV_{sunflower}$ = variety sunflower oil; $V_{instrument}$ = measurements on second, identically constructed FT-MIR instrument; CI = confidence interval; ED = Euclidian distance; *k*-NN = *k*-nearest neighbour; LOF = local outlier factor; KDE = kernel density estimation; * measurements between CI at 95.5 % and CI 99.7 %.

Table S14: Results of outlier detection for the variations of QC sample, considered separately for CI 95.5 % and CI 99.7 %; calculations based on a defined pre-period (n = 20, m = 3) of QC sample.

generated as	sample name	below CI 95.5 % inlier [%]				CI 95.5 % warning limit* suspect measurements [%]				above CI 99.7 % action limit outlier [%]			
		ED	*k*-NN	LOF	KDE	ED	*k*-NN	LOF	KDE	ED	*k*-NN	LOF	KDE
inlier	$V_{standard}$	92	100	78	95	8	0	0	5	0	0	22	0
	$V_{operator}$	100	100	80	100	0	0	0	0	0	0	20	0
outlier	$VT_{20°C}$	0	0	0	0	0	0	0	0	100	100	100	100
	$VT_{25°C}$	0	0	0	11	22	11	11	22	78	89	89	67
	$VT_{35°C}$	0	0	0	0	0	0	0	0	100	100	100	100
	$VT_{40°C}$	0	0	0	0	0	0	0	0	100	100	100	100
	VS_{7d}	100	100	90	100	0	0	0	0	0	0	10	0
	VS_{14d}	100	100	100	100	0	0	0	0	0	0	0	0
	VS_{21d}	40	70	30	70	60	30	0	30	0	0	70	0
	VS_{28d}	60	60	60	60	30	30	10	30	10	10	30	10
	$VV_{rapeseed}$	0	0	0	0	0	0	0	0	100	100	100	100
	$VV_{pumpkin}$	0	0	0	0	10	10	10	20	90	90	90	80
	$VV_{sunflower}$	0	0	0	0	0	0	0	0	100	100	100	100
	$V_{instrument}$	0	0	0	0	0	0	0	0	100	100	100	100

Variation of $V_{standard}$ = Measurement over a time period of 36 months using standard parameters; $V_{operator}$ = operator using standard parameters; VT = temperature; VS = storage (26.0°C ± 0.5°C); $VV_{rapeseed}$ = variety rapeseed oil; $VV_{pumpkin}$ = variety pumpkin seed oil, $VV_{sunflower}$ = variety sunflower oil; $V_{instrument}$ = measurements on second, identically constructed FT-MIR instrument; CI = confidence interval; ED = Euclidian distance; *k*-NN = *k*-nearest neighbour; LOF = local outlier factor; KDE = kernel density estimation; * measurements between CI at 95.5 % and CI 99.7 %.

Table S15: Results of outlier detection for the variations of QC sample, considered separately for CI 95.5 % and CI 99.7 %; calculations based on a defined pre-period (n = 30, m = 3) of QC sample.

generated as	sample name	below CI 95.5 % inlier [%]				CI 95.5 % warning limit* suspect measurements [%]				above CI 99.7 % action limit outlier [%]			
		ED	*k*-NN	LOF	KDE	ED	*k*-NN	LOF	KDE	ED	*k*-NN	LOF	KDE
inlier	$V_{standard}$	92	89	92	96	4	7	4	4	4	4	4	0
	$V_{operator}$	90	90	90	90	10	10	10	10	0	0	0	0
outlier	$VT_{20°C}$	0	0	0	0	0	0	0	0	100	100	100	100
	$VT_{25°C}$	0	0	0	0	22	11	11	22	78	89	89	78
	$VT_{35°C}$	0	0	0	0	0	0	0	10	100	100	100	90
	$VT_{40°C}$	0	0	0	0	0	0	0	0	100	100	100	100
	VS_{7d}	100	100	100	100	0	0	0	0	0	0	0	0
	VS_{14d}	100	100	100	100	0	0	0	0	0	0	0	0
	VS_{21d}	100	70	90	90	0	30	10	10	0	0	0	0
	VS_{28d}	70	20	60	40	20	70	30	50	10	10	10	10
	$VV_{rapeseed}$	0	0	0	0	0	0	0	0	100	100	100	100
	$VV_{pumpkin}$	0	0	0	0	0	0	0	0	100	100	100	100
	$VV_{sunflower}$	0	0	0	0	0	0	0	0	100	100	100	100
	$V_{instrument}$	0	0	0	0	0	0	0	0	100	100	100	100

Variation of $V_{standard}$ = Measurement over a time period of 36 months using standard parameters; $V_{operator}$ = operator using standard parameters; VT = temperature; VS = storage (26.0°C ± 0.5°C); $VV_{rapeseed}$ = variety rapeseed oil; $VV_{pumpkin}$ = variety pumpkin seed oil, $VV_{sunflower}$ = variety sunflower oil; $V_{instrument}$ = measurements on second, identically constructed FT-MIR instrument; CI = confidence interval; ED = Euclidian distance; *k*-NN = *k*-nearest neighbour; LOF = local outlier factor; KDE = kernel density estimation; * measurements between CI at 95.5 % and CI 99.7 %.

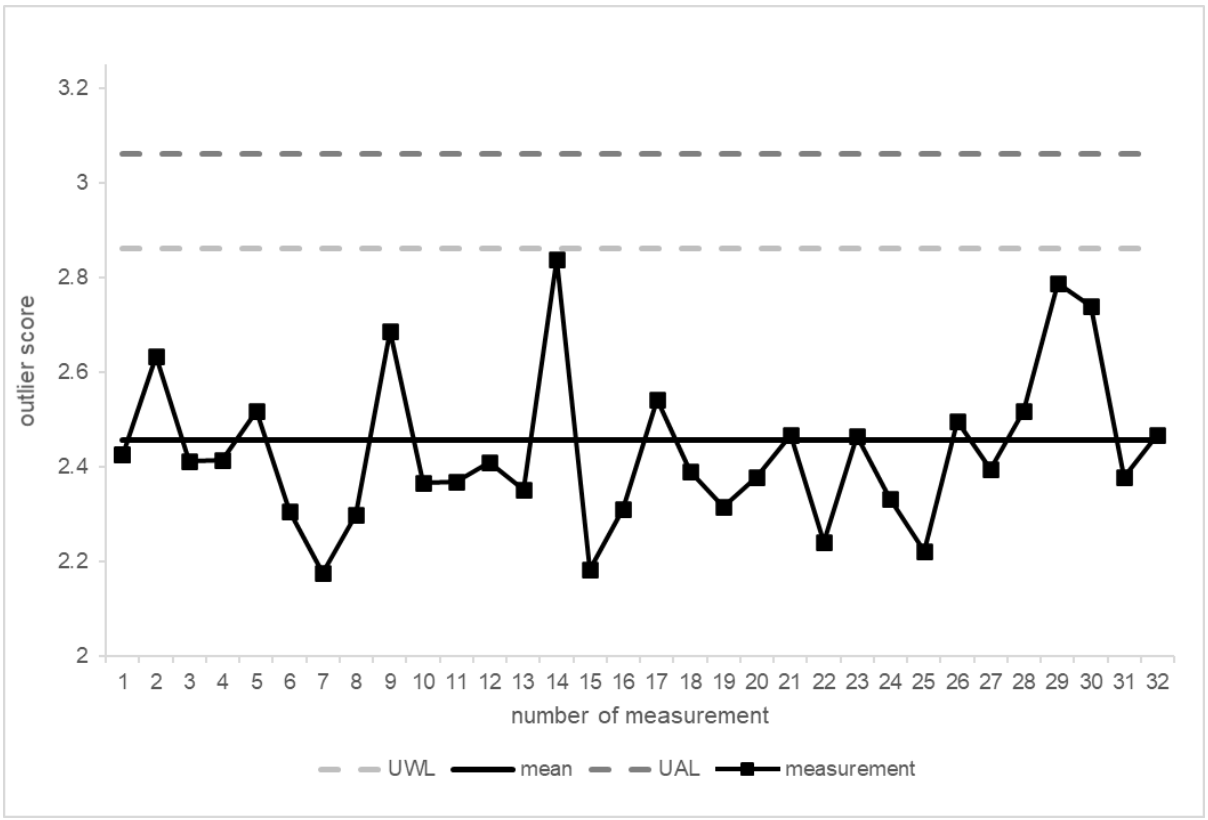

Figure S1: Mean value control chart based on *k*-NN method and pre-period (n = 25, m = 3); dashed light grey line - upper warning limit (UWL); dashed dark grey line - upper action limit (UAL); black, horizontal line - mean of calculated outlier scores for pre-period measurements; black line with black squares - measurements of $V_{standard}$ (n = 32).

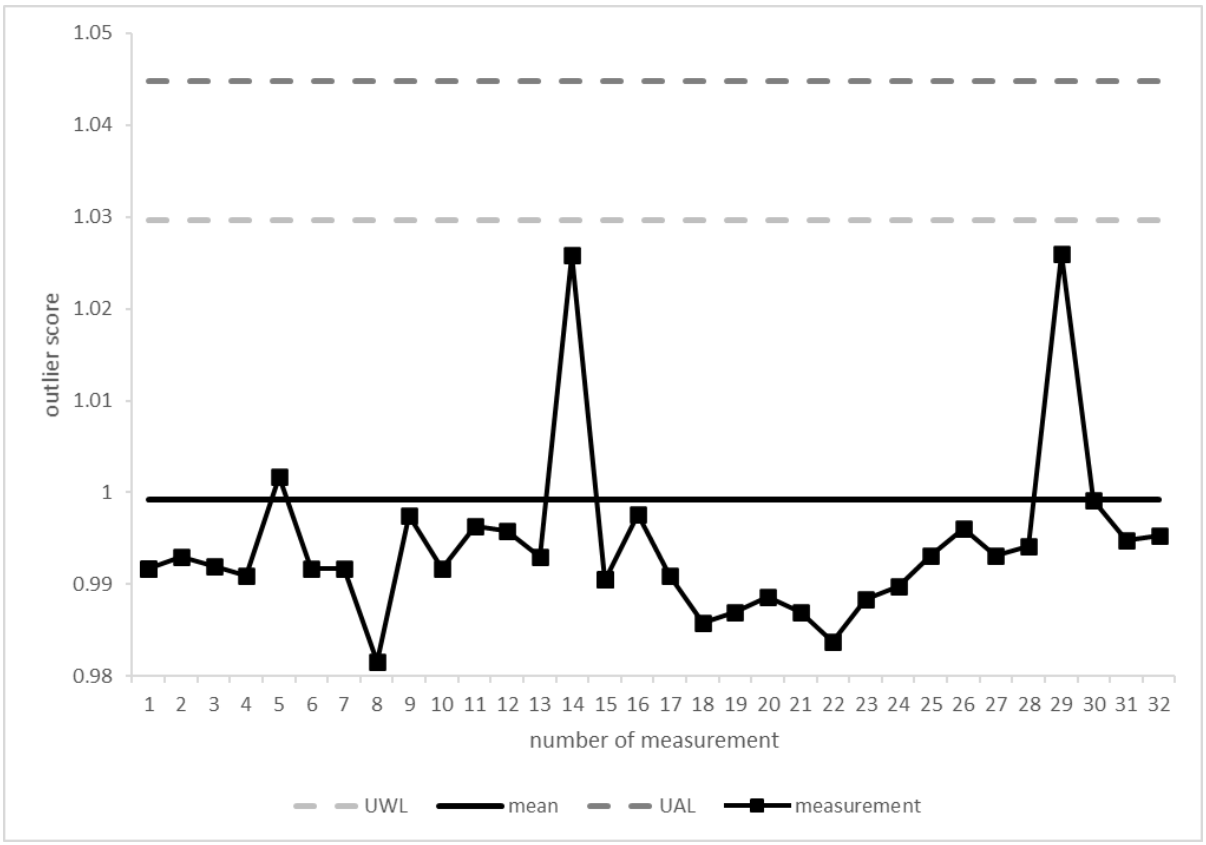

Figure S2: Mean value control chart based on local outlier factor method and pre-period (n = 25, m = 3); dashed light grey line - upper warning limit (UWL); dashed dark grey line - upper action limit (UAL); black, horizontal line - mean of calculated outlier scores for pre-period measurements; black line with black squares - measurements of $V_{standard}$ (n = 32).

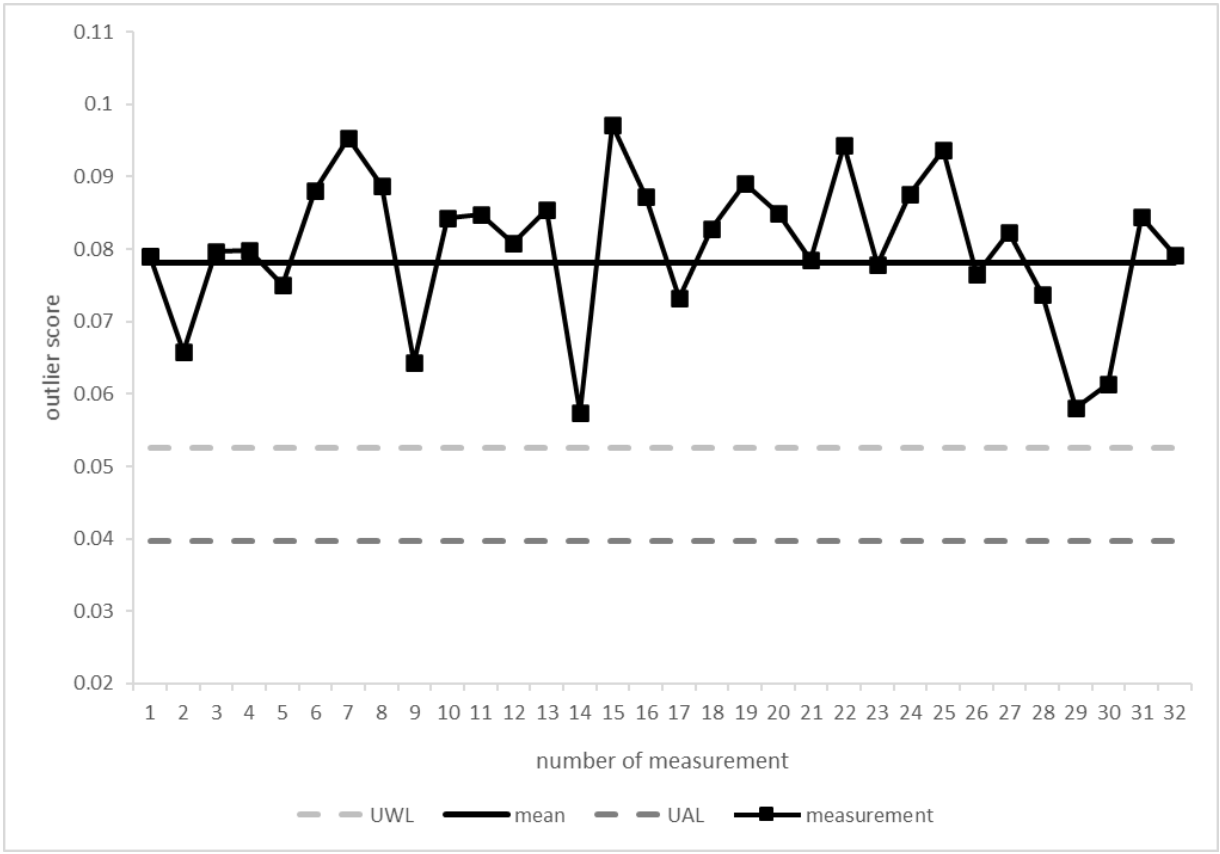

Figure S3: Mean value control chart based on kernel density estimation method and pre-period (n = 25, m = 3); dashed light grey line - upper warning limit (UWL); dashed dark grey line - upper action limit (UAL); black, horizontal line - mean of calculated outlier scores for pre-period measurements; black line with black squares - measurements of $V_{standard}$ (n = 32).

10.3 Ergänzende Informationen zur Publikation „*Towards common useable spectra in non-targeted analysis - a feasibility study by mid-infrared spectroscopy, transfer and correction approaches*“ in der Fachzeitschrift *Chemometrics and Intelligent Laboratory Systems*

Table S8: Sample set of edible seed oils with different varieties; pumpkin seed oil n = 44, rapeseed oil n = 40, sunflower oil = 3, linseed oil = 3.

Variety	Type of processing	Organic agriculture	Country of origin
pumpkin seed oil	cold pressed	yes	EU-/Non-EU-agriculture
pumpkin seed oil	cold pressed	yes	Austria
pumpkin seed oil	refined	no	Austria
pumpkin seed oil	refined	no	no information
pumpkin seed oil	refined	no	Austria
pumpkin seed oil	refined	yes	Austria
pumpkin seed oil	refined	yes	Germany
pumpkin seed oil	refined	yes	Austria
pumpkin seed oil	refined	no	Austria
pumpkin seed oil	refined	no	no information
pumpkin seed oil	virgin	yes	Germany
pumpkin seed oil	refined	yes	Hungary
pumpkin seed oil	refined	yes	Austria
pumpkin seed oil	virgin	yes	Germany
pumpkin seed oil	refined	yes	Austria
pumpkin seed oil	refined	yes	Austria
pumpkin seed oil	refined	no	no information
pumpkin seed oil	refined	no	no information
pumpkin seed oil	refined	no	Austria
pumpkin seed oil	cold pressed	yes	Non-EU-agriculture
pumpkin seed oil	refined	yes	Austria
pumpkin seed oil	refined	yes	EU-agriculture

pumpkin seed oil	refined	yes	Austria
pumpkin seed oil	refined	yes	Austria
pumpkin seed oil	refined	yes	Austria
pumpkin seed oil	refined	no	EU-/Non-EU-agriculture
pumpkin seed oil	refined	yes	Austria
pumpkin seed oil	cold pressed	yes	Austria
pumpkin seed oil	refined	no	Austria
pumpkin seed oil	refined	no	no information
pumpkin seed oil	refined	no	Austria
pumpkin seed oil	refined	yes	Austria
pumpkin seed oil	cold pressed	yes	Austria
pumpkin seed oil	cold pressed	no	Austria
pumpkin seed oil	refined	no	Austria
pumpkin seed oil	refined	no	Austria
pumpkin seed oil	refined	yes	Austria
pumpkin seed oil	refined	yes	EU-agriculture
pumpkin seed oil	cold pressed	yes	Austria
pumpkin seed oil	virgin	yes	EU-agriculture
rapeseed oil	refined	no	Germany
rapeseed oil	cold pressed	no	Germany
rapeseed oil	refined	no	Germany
rapeseed oil	virgin	no	no information
rapeseed oil	cold pressed	no	Germany
rapeseed oil	virgin	no	Germany
rapeseed oil	refined	no	no information
rapeseed oil	refined	no	Austria
rapeseed oil	refined	no	no information
rapeseed oil	virgin	no	no information
rapeseed oil	virgin	yes	EU-agriculture
rapeseed oil	cold pressed	yes	EU-/Non-EU-agriculture
rapeseed oil	refined	no	Germany
rapeseed oil	virgin	no	Germany

rapeseed oil	cold pressed	no	Germany
rapeseed oil	cold pressed	no	Germany
rapeseed oil	cold pressed	no	Germany
rapeseed oil	refined	no	no information
rapeseed oil	virgin	no	Germany
rapeseed oil	refined	no	Austria
rapeseed oil	refined	no	no information
rapeseed oil	refined	no	Germany
rapeseed oil	cold pressed	no	Germany
rapeseed oil	virgin	no	Germany
rapeseed oil	refined	no	no information
rapeseed oil	refined	no	no information
rapeseed oil	refined	no	no information
rapeseed oil	virgin	no	Germany
rapeseed oil	virgin	yes	EU-agriculture
rapeseed oil	cold pressed	no	Germany
rapeseed oil	cold pressed	yes	EU-agriculture
rapeseed oil	refined	no	Germany
rapeseed oil	virgin	yes	EU-agriculture
rapeseed oil	virgin	yes	France
rapeseed oil	virgin	yes	EU-agriculture
rapeseed oil	refined	no	no information
rapeseed oil	refined	no	no information
rapeseed oil	refined	no	no information
rapeseed oil	refined	no	no information
rapeseed oil	refined	no	no information
rapeseed oil	refined	no	no information
rapeseed oil	refined	no	no information
rapeseed oil	refined	no	no information
rapeseed oil	refined	no	no information
sunflower oil	cold pressed	no	no information
sunflower oil	refined	no	no information
sunflower oil	refined	no	no information
linseed oil	cold pressed	no	Germany

linseed oil	cold pressed	no	Germany
linseed oil	cold pressed	no	Germany

Table S2: Applied pre-processing combinations for improvement of spectra comparability of the instruments.

PP	1st step	2nd step	3rd step
1	SNV	Mean center	
2	SNV	1st derivative, SG	Mean center
3	SNV	2nd derivative, SG	Mean center
4	Normalization	Mean Center	
5	Normalization	1st derivative, SG	Mean Center
6	Normalization	2nd derivative, SG	Mean Center
7	MSC (median)	Mean Center	
8	MSC (median)	1st derivative, SG	Mean Center
9	MSC (median)	2nd derivative, SG	Mean Center

PP = pre-processing; SNV = standard normal variate; SG = Savitzky-Golay polynomial derivative filter (1st and 2nd order and centred 11 point window); MSC = Multiplicative scatter correction.

Table S3: Equations for calculating the performance parameters.

Parameter	Equation
Sensitivity [%]	$\frac{TP}{TP + FN}$
Specificity [%]	$\frac{TN}{FP + TN}$
Accuracy [%]	$\frac{TP + TN}{TP + FN + FP + TN}$
Precision [%]	$\frac{TP}{TP + FP}$
Classification error [%]	$\frac{(100\text{-}Sen) + (100\text{-}Spec)}{2}$

TP = True Positive, TN = True Negative, FP = True Positive, FN = False Negative, Sen = Sensitivity, Spec = Specificity.

Table S4: Classification results of PLS-DA predictions, with MIR 1 spectral data used for model building, and original spectral data from MIR 2 and 3 used for system challenge.

	MIR 2	MIR 3
Sensitivity [%]	20	0
Specificity [%]	100	100
Accuracy [%]	60	50
Precision [%]	11	0
RMSEP	1.006	1.353

RMSEP = root mean square error of prediction.

Table S5: Correlation plot for the determination of a correction factor based on absorbances of QC sample measurements (n = 15, m = 3), acquired on MIR 1 and MIR 2, grey dotted line shows the linear regression with y = 0.8771 and R^2 = 0.998.

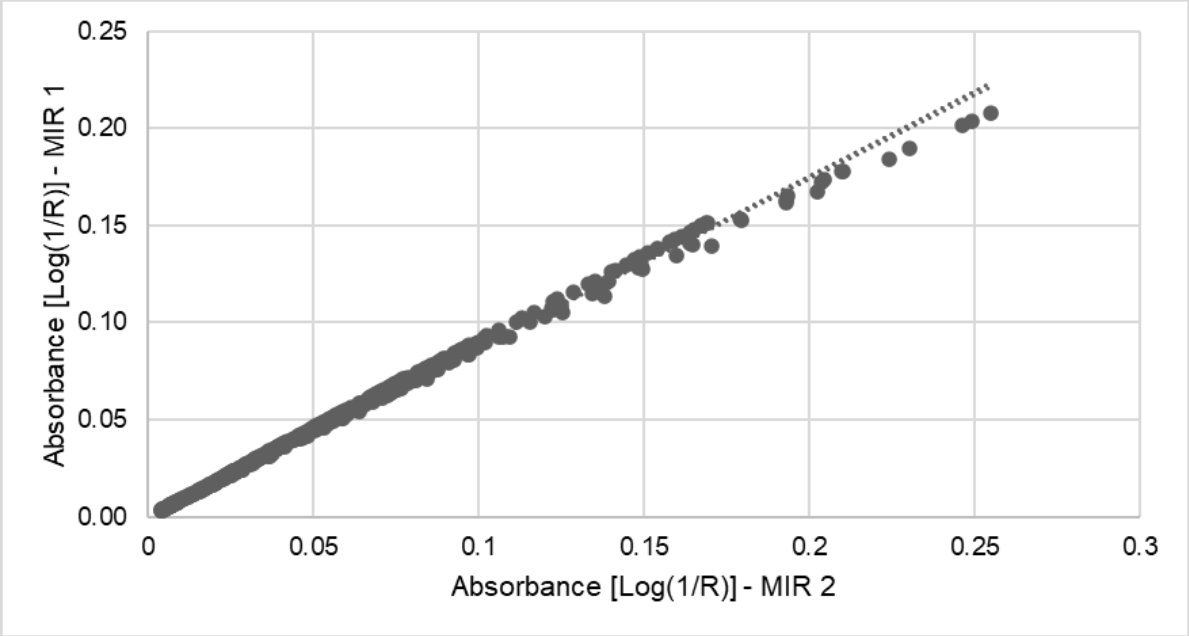

Table S6: Correlation plot for the determination of a correction factor based on absorbances of QC measurements (n = 15, m = 3), measured on MIR 1 and MIR 3, grey dotted line shows the linear regression with y = 0.881 and R^2 = 0.997.

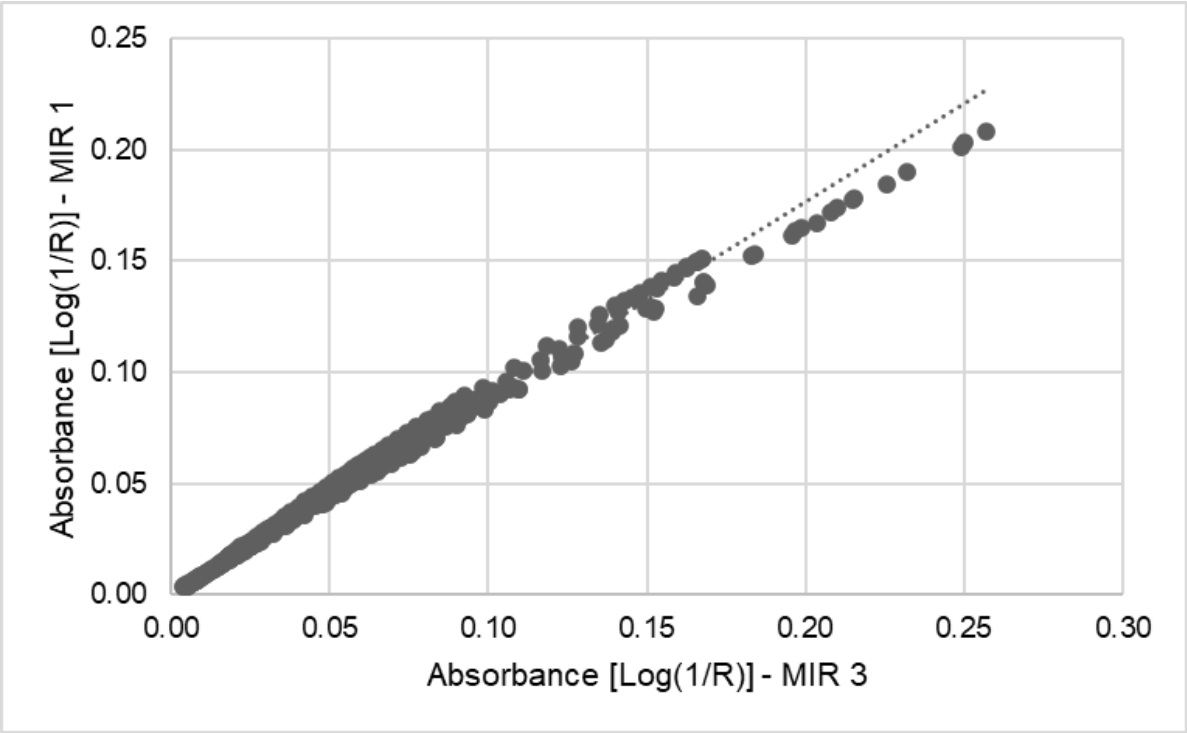

Table S7: PLS-DA results for various pre-processing steps on the MIR 2 and 3 data.

				Parameter			
PP	**Instrument**	Latent variables	Cumu-lative variance	Sensitivity [%]	Specificity [%]	Accuracy [%]	Precision [%]
1	MIR 2 MIR 3	5	91	0	100 100	50	0
2	MIR 2 MIR 3	4	94		100		
3	MIR 2 MIR 3	3	92		100		
4	MIR 2 MIR 3	5	94	0	100 100	50	0
5	MIR 2 MIR 3	4	95		100		
6	MIR 2	3	94		100		

7	MIR 3 MIR 2 MIR 3	6	96	0	100	50	0
8	MIR 2 MIR 3	5 4	96 95		100		
9	MIR 2 MIR 3	3	92		100		

PP = pre-processing; SNV = standard normal variate; SG = Savitzky-Golay polynomial derivative filter (1st and 2nd order and centred 11 point window); MSC = Multiplicative scatter correction; 1: SNV, mean center; 2: SNV, 1st derivative, SG, mean center; 3: SNV, 2nd derivative, SG, mean center; 4: normalization, mean center; 5: normalization, 1st derivative, mean center; 6: normalization, 2nd derivative, mean center; 7: MSC, mean center; 8: MSC, 1st derivative, mean center; 9: MSC, 2nd derivative, mean center.

11 Curriculum Vitae

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04/2024 -	Referentin am Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, Berlin, Deutschland
09/2021 - 03/2024	*Project Officer* am *Joint Research Center*, Geel, Belgien
01/2020 - 09/2021	Referentin am Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, Berlin, Deutschland
12/2016 - 12/2019	Doktorandin am Bundesinstitut für Risikobewertung, Berlin, Deutschland; akademische Betreuung durch Prof. Dr. Glomb, Institut für Chemie – Lebensmittelchemie und Umweltchemie, Martin-Luther-Universität Halle-Wittenberg, Deutschland
01/2019	Forschungsaufenthalt am Walloon *Agricultural Research Centre* (CRA-W) in der Fachgruppe „*Food and Feed Quality*“, Gembloux, Belgien
08/2015 - 11/2016	wissenschaftliche Mitarbeiterin im Landeslabor Berlin-Brandenburg, Berlin, Deutschland
04/2014 - 03/2015	3. Prüfungsabschnitt der Staatsprüfung für LebensmittelchemikerInnen am Landeslabor Berlin-Brandenburg, Berlin, Deutschland

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10/2007 - 09/2010	Studium der Lebensmittelchemie an der Technischen Universität Berlin, Deutschland (1. Prüfungsabschnitt der Staatsprüfung für LebensmittelchemikerInnen)
09/1998 – 06/2007	Herder-Gymnasium (Allgemeine Hochschulreife), Greifswald, Deutschland

12 Eigenständigkeitserklärung

Hiermit versichere ich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt habe und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Weiterhin versichere ich, dass ich keine vergeblichen Promotionsversuche unternommen habe und die Dissertation in der gegenwärtigen bzw. in einer anderen Fassung keiner anderen Fakultät vorgelegen hat.

Berlin, den 18.07.2024