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**Physicochemical, thermal, and spectroscopic characterisation
of oils from berry seed by-products
in terms of authenticity assessment**

Charakterystyka fizykochemiczna, termiczna i spektroskopowa olejów
z produktów ubocznych nasion owoców w kontekście oceny autentyczności

Doctoral dissertation in the field of agricultural science
in the discipline of food technology and nutrition

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'Two more years: then I'll get my diploma and I'm done.'
'No... don't ever stop: I'll give you the money, you should keep studying.'
I gave a nervous laugh, then said, 'Thanks, but at a certain point school is over.'
'Not for you: you're my brilliant friend, you have to be the best of all, boys and girls.'

— Elena Ferrante, *My Brilliant Friend*

This work is dedicated to my brilliant friends,

My late **grandma**,
who passed away a day before my PhD entrance exam.

My **mom**,
who always dreamed of pursuing post-graduate studies.

Marcellus Arnold,
who always been by my side from the beginning.

My sincere gratitude to my supervisor, **prof. dr hab. Jolanta Tomaszewska-Gras**.
The past four years went by so quickly, I will always remember
the grapes from your parents' garden and your homemade cherry pierogis.

List of scientific papers

No	Publications	IF (year)	MEiN (year)
P1	Rajagukguk, Y. V. , Islam, M., & Tomaszewska-Gras, J. (2023). Influence of Seeds' Age and Clarification of Cold-Pressed Raspberry (<i>Rubus idaeus</i> L.) Oil on the DSC Oxidative Stability and Phase Transition Profiles. <i>Foods</i> , 12(2), 358. https://doi.org/10.3390/foods12020358	4.7	140
P2	Rajagukguk, Y. V. , Islam, M., Grygier, A., & Tomaszewska-Gras, J. (2023). Thermal and spectroscopic profiles variation of cold-pressed raspberry seed oil studied by DSC, UV/VIS, and FTIR techniques. <i>Journal of Food Composition and Analysis</i> , 124, 105723. https://doi.org/10.1016/j.jfca.2023.105723	4.0	100
P3	Rajagukguk, Y. V. , Islam, M., Grygier, A., Siger, A., Rudzińska, M., & Tomaszewska-Gras, J. (2024). Physicochemical characteristics of cold-pressed blackcurrant-, strawberry-, and raspberry seed oils during storage and its influence on thermo-oxidative stability. <i>NFS Journal</i> , 37, 100195. https://doi.org/10.1016/j.nfs.2024.100195	3.3	140
P4	Rajagukguk, Y. V. , Cevoli, C., Grigoletto, I., & Tomaszewska-Gras, J. (2024). Rapid determination of the storage time of cold-pressed berry seed oils using flash gas chromatography E-Nose coupled with chemometrics. <i>Journal of Food Engineering</i> , 364, 111795. https://doi.org/10.1016/j.jfoodeng.2023.111795	5.8	140
P5	Rajagukguk, Y. V. , & Tomaszewska-Gras, J. (2025). Advanced authenticity screening of commercial berry seed oils using full FTIR spectra and DSC curves coupled with chemometrics. <i>LWT</i> , 117680. https://doi.org/10.1016/j.lwt.2025.117680	6.6	100
P6	Rajagukguk, Y.V. , Ryszczńska S., Grygier A., Siger A., Waśkiewicz A., & Tomaszewska-Gras, J. (2025). Data fusion of DSC melting curves and FTIR spectra in relation to physicochemical properties for distinguishing berry seed oils by various extraction methods (under review in <i>Journal of Food Composition Analysis</i> since 18.05.2025)	-	-
Total		24.4	620

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List of abbreviations

Analytical instruments

DSC	Differential scanning calorimetry
FTIR	Fourier transform Infrared
GC	Gas chromatography
HPLC	High pressure liquid chromatography

Data analysis

PCA	Principal component analysis
PLS	Partial least square
(PLS)-R	(...)- regression
DA	Discriminant analysis
O-(PLS-DA)	Orthogonal-(...)
PCA	Principal component analysis
SMLR	Stepwise multiple linear regression
LV	Latent variable
RMSE	Root mean square
(RMSE)-CV	(...)-Cross validation
(RMSE)-P	(...)-Prediction
VIP	Variable importance in projection
SIMCA	Soft independent modelling by class analogy
HCA	Hierarchical cluster analysis

Samples

BSO	Berry seed oils
BC	Blackcurrant seed oils
RB	Raspberry seed oils
ST	Strawberry seed oils

Compounds

FA	Fatty acid
U-(FA)	Unsaturated-(...)
S-(FA)	Saturated-(...)

Analyses

AV	Acid value
p-AnV	p-anisidine value
PV	Peroxide value
FFA	Free fatty acid
OIT	Oxidation induction time
T _m	Melting peak temperature
H _m	Melting peak enthalpy
h _m	Melting peak height

Summary

The demand for berry seed oils as food supplement, nutraceutical, and cosmetic products were observed in the recent years. Despite the growing interest, consumers remain vulnerable to fraud due to the lack of specific regulation for berry seed oils. To address the gap, the main aim of this work was to develop rapid and reliable methods for assessing the authenticity of berry seed oils based on their physicochemical, thermal, and spectroscopic profiles. The pioneering nature of this work lies in using the entire dataset of DSC and FTIR profiles as untargeted analytical methods to address significant authenticity issues related to oil freshness, adulteration, and label transparency.

This study was divided into three main stages: method optimisation of thermal and spectroscopic techniques, physicochemical characterisation of fresh and stored oils, and authenticity assessment of berry seed oils. In the first stage of research, it was found that lower seed quality and the clarification process of oil negatively affected oxidative stability and antioxidative properties. However, no differences in DSC phase transition profiles were observed in relation to the clarification process. The scanning rate affected the melting and crystallization DSC profiles of oils, and the melting profile obtained at a scanning rate of 5 °C/min was considered the most useful for further authenticity studies of the oils. Among the spectroscopic methods, FTIR was considered more informative than UV-VIS spectroscopy for further studies on the discrimination of oils. Using the optimised methods, three main authenticity issues were addressed: oil freshness, commercial oil screening, and label clarity regarding the declaration of extraction methods.

The physicochemical characteristics revealed distinct properties of each type. Blackcurrant seed oils had the higher content of carotenoids, chlorophylls and γ -linolenic acid, while raspberry seed oils exhibited the highest antioxidant activity (DPPH), tocopherols content and thermo-oxidative stability (OIT). In turn strawberry seed oils contained the highest amount of α -linolenic acid. All berry seed oils were thermally stable during one year of storage, as indicated by the OIT index, despite the reduction of antioxidants and deterioration in quality.

The storage time of oils was determined by assessing changes in their volatile profiles using Flash GC E-Nose coupled with PLSR models. During commercial oil screening, optimised PLS-DA models based on DSC and FTIR data performed well in classifying oils according to their botanical origins. The DSC and FTIR dataset complemented each other in presenting typical characteristics of berry seed oils from different extraction methods. A novel approach of low-level data fusion from both DSC and FTIR datasets was implemented, creating

a fused dataset referred to as “DSC-FTIR”. The fused dataset was used for rapid classification with the SIMCA model. This model from DSC-FTIR dataset outperformed models built based on single analytical methods in classifying berry types and extraction methods.

This work provides essential information about developing untargeted authenticity assessment methods specifically for berry seed oils. The knowledge gained from this study can serve as a foundation for food policy makers to establish the regulatory standards for berry seed oils.

Keywords: Berry seed oils, authenticity, differential scanning calorimetry (DSC, Fourier transform infrared spectroscopy (FTIR), chemometrics.

Streszczenie

W ostatnich latach znacznie wzrósł popyt na oleje z nasion owoców jagodowych jako suplementy diety, nutraceutyki i produkty kosmetyczne. Pomimo rosnącego zainteresowania, konsumenci pozostają narażeni na oszustwa z powodu braku szczegółowych regulacji dotyczących tych olejów. W celu rozwiązania tej luki, głównym celem niniejszej pracy było opracowanie szybkich i wiarygodnych metod oceny autentyczności olejów z nasion jagodowych na podstawie ich właściwości fizykochemicznych, termicznych i spektroskopowych. Pionierski charakter tej pracy polega na zastosowaniu całych profili DSC i FTIR jako niecelowanych metod analitycznych, mających na celu rozwiązanie istotnych problemów związanych z autentycznością olejów, takich jak świeżość, fałszowanie i przejrzystość etykiet.

Badanie zostało podzielone na trzy główne etapy: optymalizację metod termicznych i spektroskopowych, fizykochemiczną charakterystykę olejów świeżych i przechowywanych oraz ocenę autentyczności olejów z nasion owoców jagodowych. W pierwszym etapie badania stwierdzono, że niższa jakość nasion oraz proces klarowania oleju negatywnie wpływały na stabilność oksydacyjną i właściwości antyoksydacyjne. Jednakże, nie zaobserwowano żadnych różnic w profilach przejść fazowych DSC w zależności od procesu klarowania. Szybkość skanowania miała wpływ na profile topnienia i krystalizacji DSC olejów, a profil topnienia uzyskany przy szybkości skanowania 5°C/min uznano za najbardziej przydatny do dalszych badań autentyczności olejów. Wśród metod spektroskopowych FTIR uznano za bardziej przydatną niż spektroskopię UV-VIS w kontekście dalszych badań służących do rozróżniania olejów. Z wykorzystaniem zoptymalizowanych metod rozwiązano trzy główne problemy związane z autentycznością: świeżość oleju, screening olejów komercyjnych oraz przejrzystość etykiet w odniesieniu do deklaracji metod ekstrakcji.

Badania fizykochemicznych właściwości wykazały charakterystyczne właściwości dla każdego typu oleju. Oleje z nasion czarnej porzeczki charakteryzowały się wyższą zawartością karotenoidów, chlorofili i kwasu γ -linolenowego, podczas gdy oleje z nasion malin wykazywały najwyższą aktywność antyoksydacyjną (DPPH), zawartość tokoferoli oraz stabilność termo-oksydacyjną (OIT). Z kolei oleje z nasion truskawek zawierały najwyższą ilość kwasu α -linolenowego. Wszystkie oleje z nasion owoców jagodowych były termicznie stabilne podczas 1 roku przechowywania, co zostało potwierdzone przez wskaźnik OIT, mimo obniżenia zawartości antyoksydantów i wzrostu liczb tłuszczowych. Określenie czasu

przechowywania olejów było możliwe dzięki ocenie zmian profili związków lotnych za pomocą Flash GC E-Nose połączonej z modelami PLSR.

W ramach screeningu olejów komercyjnych, zoptymalizowane modele PLS-DA oparte na danych DSC i FTIR wykazały wysoką skuteczność w klasyfikacji olejów według ich pochodzenia botanicznego. Zestawy danych DSC i FTIR wzajemnie się uzupełniały w prezentowaniu charakterystycznych cech olejów z nasion owoców jagodowych, otrzymanych za pomocą różnych metod ekstrakcji. Zastosowano nowatorskie podejście w postaci fuzji danych pierwszego poziomu z obu zestawów danych DSC i FTIR, tworząc nowy zestaw danych nazywany „DSC-FTIR”. Fuzja danych została wykorzystana do szybkiej klasyfikacji za pomocą modelu SIMCA. Model ten oparty na zestawie danych DSC-FTIR przewyższył modele oparte na pojedynczych metodach analitycznych w klasyfikacji typów olejów jak i metod ekstrakcji.

Niniejsza praca dostarcza istotnych informacji na temat opracowywania niecelowanych metod oceny autentyczności, szczególnie dla olejów z nasion owoców jagodowych. Wiedza zdobyta w ramach tego badania może stanowić podstawę dla twórców prawa żywnościowego do opracowania regulacji dla olejów z nasion owoców jagodowych.

Słowa kluczowe: oleje z nasion owoców jagodowych, autentyczność, różnicowa kalorymetria skaningowa (DSC), spektroskopia w podczerwieni z transformatą Fouriera (FTIR), chemometria

1. Introduction

World's production of fruits increases annually; the production generates a growing problem related to the management and the accumulation of agro-industrial waste. Approximately 20% of the global food waste consist of the by-products from fruit processing, such as pomace, peels, roots, or seeds (FAO, 2019). Responding to the waste management problem, a sustainable resource management called circular economy was introduced to replace the conventional linear-economy model. Circular economy prioritises the minimisation of waste production by utilising the by-products from one process as a raw materials for others (Przykaza et al., 2021). The circular economy concept was also implemented in one of European Union's work plan, namely The European Green Deal, under the slogan "Turning waste into resources" (European Commission, 2019). From food industry perspective, reducing the amount of food waste and the valorisation of by-products will support the goal of circular economy. Some barriers in the implementation of circular economy includes lack of policies to support circular economy transition and lack of proven technologies (Kirchherr et al., 2018). Hence, promoting the by-product utilisation, sustainable production, and supporting the development of regulation related to oils from by-products are a relevant approach to reach the goal for circular economic growth in European Union.

Berry seed oil is one of the valorised goods from berries by-products. The demand for this oil as food supplement, nutraceutical, and cosmetic products were observed in the recent years. Despite the growing interest, the consumer remains unprotected from fraud. Being a niche oil with lack of information regarding its authentic properties, berry seed oils regulation is nowhere to be found in the existing regulation (i.e. Codex Alimentarius, EEC 2568/91) as it only covers the general characteristics of widely consumed oil. At the same time, oil adulteration remains an escalating challenge in European Union, this concern was marked with a consistent increase in edible oil's fraud prevalence from 15 reported cases in first quarter of 2018 to 182 cumulative cases from 2023 until 2025 (Joint Research Centre, 2025). Such cases do not solely cause financial loses but also poses public health risk. As the oil adulteration techniques becoming increasingly sophisticated, progressive scientific efforts to confront the vulnerabilities were needed.

Edible oil can be guaranteed as authentic when the oil is pure, appropriately labelled with respect to its physicochemical attributes, and meet the criteria established by regulatory organisations such as Codex Alimentarius Commission. In the recent report on suspected agri-food frauds in the EU, cases were categorised into three main types: record tampering, product

tampering, and other non-compliances (including implicit claim violations) (Joint Research Centre, 2025). Record tampering includes false health claims, product information, and method of production. Product tampering covers the aspects of adulteration, substitution with inferior oils, additives, and undeclared process. While the presence of chemical residue, unauthorised origins, and food ingredients not allowed in EU were placed in the last category with the highest prevalences.

In the case of seed oils, authenticity assessments based on calorimetry and spectroscopy techniques have been gradually developed (Islam et al., 2022; Ye and Meng, 2022). Differential scanning calorimetry (DSC) is used as the primary calorimetric instrument to assess seed oil authenticity based on melting or crystallization curves, as well as the change in oxidative stability. Recent knowledge about the feasibility of DSC for authenticity assessment were reported on the classification study of olive oil from different commercial categories (i.e. extra virgin, virgin, and lampante olive oil), classification of butterfat based on seasonal variability (i.e. summer, winter) (Tomaszewska-Gras, 2016a), and the detection of the adulterants in milk (Chiavaro et al., 2009; Farah et al., 2021). These studies also highlighted general advantages of DSC techniques including short analysis time, environmentally friendliness, and low setup requirements. Even though fundamental information on DSC thermal profiles in berry seed oils was reported over a decade ago (Oomah et al., 2000), no specific study has been conducted on the authenticity assessment based on thermal profiles of berry seed oils.

To complement the thermal analysis, the presented study also used Fourier transform mid-infrared (FTIR) for authenticity study in berry seed oils. This technique provides information about biochemical compound within defined spectral ranges. Both DSC and FTIR were classified as “non-targeted” technique, as it investigated general composition and properties rather than detecting a specific compound in food matrices. The results from non-targeted authenticity instruments are often coupled with multivariate data analysis to enhance the data interpretation. Previous works reported the effectiveness of coupling thermal and spectroscopic dataset with chemometrics, such as in discriminating almond’s cultivar (Sanahuja et al., 2011), evaluating lipid degradation in olive oil (Chiavaro et al., 2013), and determining the geographical and temporal origins of crude palm oil (Rozali et al., 2023).

The FTIR spectra of edible oils are remarkably comparable to one another, in contrast to DSC melting profiles. A recent study on pattern recognition techniques to analyse FTIR spectra from 20 edible oil types ($n = 97$) confirmed that each edible oil possessed their own distinct traits (Sota-Uba et al., 2021). Twenty edible oils were divided into four groups using hierarchical clustering analysis. According to authors, the fingerprint patterns of avocado,

peanut, almond, and sunflower oils are comparable to those of olive oils. However, other oils with distinct fingerprint patterns, like soybean, sesame, and canola, were classified into separate groups. Noting about these specific properties in each oil, it is necessary to increase the understanding of FTIR's application in other less-studied oils.

Other than untargeted analyses using thermal and spectroscopic profiles, some authenticity studies in fruit seed oils were previously carried out using various approaches such as chromatographic and metabolomic profiling by gas chromatography coupled with mass spectrometry (GC-MS), high-performance thin-layer chromatography (HPTLC), and high-performance anion-exchange chromatography with pulsed amperometry detection (HPAEC-PAD) (Krstić et al., 2022). These methods provide comprehensive information about primary metabolites belonging to polar lipids, acylglycerols, free fatty acids, sterols, saccharides, and sugar alcohols. In the case of detecting adulterants, recent work reported the study of triacylglycerol profiles of pumpkin seed oils using ultra-high performance liquid chromatography (UHPLC) coupled with quadrupole time-of-flight mass spectrometry (QTOF-MS), which allows reliable detection of adulteration down to 3% (w/w) (Schwarz et al., 2025). In contrast with the usage of these advanced analytical instruments, a straight-forward authenticity study utilised *cis*-vaccenic acid as a marker to differentiate avocado oil with other high oleic seed oils that was presumed as adulterants (Green and Wang, 2022). Such advances were made possible due to the extensive fundamental research that back up the findings, which are currently limited for berry seed oils.

Therefore, the presented study aimed to address the gap regarding the lack of knowledge about berry seed oils authenticity by developing a rapid and reliable method based on physicochemical, thermal, and spectroscopic profiles. The developed methods were used to solve prominent authenticity issues related with oil freshness and label transparency by providing practical implementation to use DSC and FTIR data combined with chemometrics. The pioneering nature of this work focuses on the effort to use full DSC curves and FTIR spectra during the authenticity assessment of berry seed oils. Expanding the utilisation of untargeted analyses will benefit the industrial laboratories which does not have any access to specific targeted instruments dedicated for authenticity assessment. Additionally, the knowledge from these works might benefits industry practitioners and regulatory organisations in developing new quality standards to ensure berry seed oils authenticity.

The research was divided into three primary stages: Optimisation of thermal and spectroscopic methods, physicochemical characterisation of authentic berry seed oils, and

authenticity assessment by employing the optimised analytical parameters to solve specific authentication issues. The research scheme diagram is presented in Fig 1.

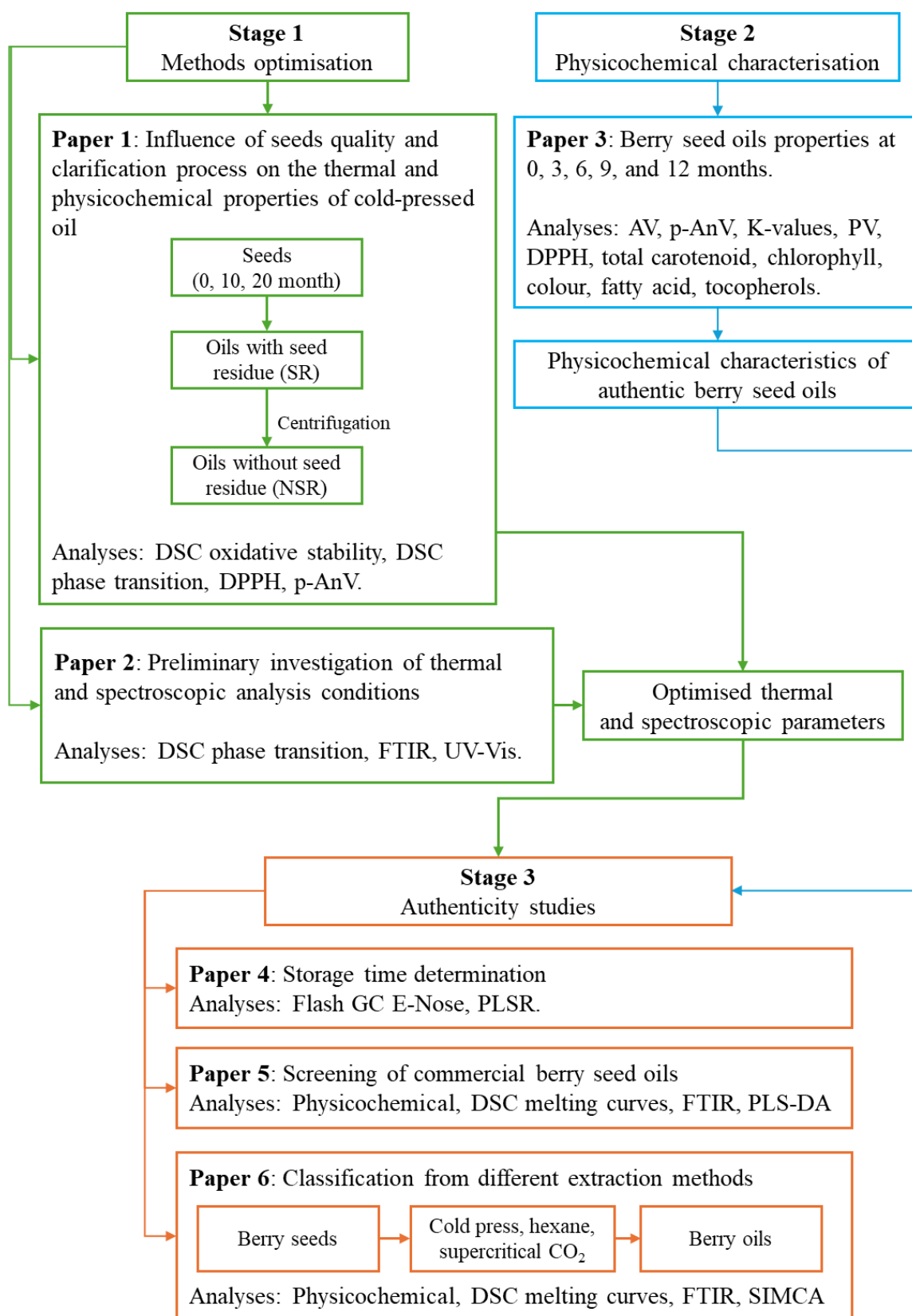


Figure 1. Diagram of the research scheme

2. Research aims and hypotheses

2.1 Aims

The main aim of this research was to develop rapid and reliable methods for assessing the authenticity of berry seed oils (raspberry, strawberry, blackcurrant) based on their physicochemical, thermal, and spectroscopic profiles. This work was built based on six papers, which were subsequently addressed as P1 to P6. To achieve the main aim, the specific objectives of the study were:

- to optimise thermal and spectroscopic methods for authenticity assessment of berry seed oils (P1, P2)
- to characterise the physicochemical properties of authentic berry seed oils (P3)
- to assess the authenticity of berry seed oils differing in storage time (P4), botanical origin from commercial samples (P5), and extraction methods (P6).

2.2 Hypotheses

Following the aims, the research hypotheses (H1 to H5) of this study were divided into the following points:

- H1: Seed freshness, clarification process (P1), and analytical conditions (P2) affected thermal and spectroscopic properties of berry seed oils.
- H2: Authentic berry seed oils possess distinctive physicochemical properties (P3) that can be used as reference markers for authenticity.
- H3: Berry seed oils differing in storage times can be distinguished through E-nose volatile profiling (P4).
- H4: Differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FTIR) are feasible for rapid authenticity screening of commercial berry seed oils (P5).
- H5: Berry seed oils from different extractions (i.e. cold-pressing, n-hexane, supercritical CO₂) exhibited distinguishable thermal and spectroscopic profiles (P6).

3. Materials and methods

3.1 Materials

Blackcurrant (*Ribes nigrum*), raspberry (*Rubus idaeus*), and strawberry (*Fragaria ananassa*) seeds were acquired from local producers, Olvita Gołuch Sp. k. (Marcinowice, Poland) and GreenField Sp. z.o.o. (Warsaw, Poland). To capture the natural variability, these

seeds were obtained from three different production batches. A total of 9 types of berry seeds (3 blackcurrant, 3 raspberries, 3 strawberries) were prepared for further analysis. Additionally, 38 commercial berry seed oils (10 blackcurrant, 18 raspberries, 10 strawberries) were also bought from the market. Before analyses, seeds and oils were stored at -80 °C in the dark.

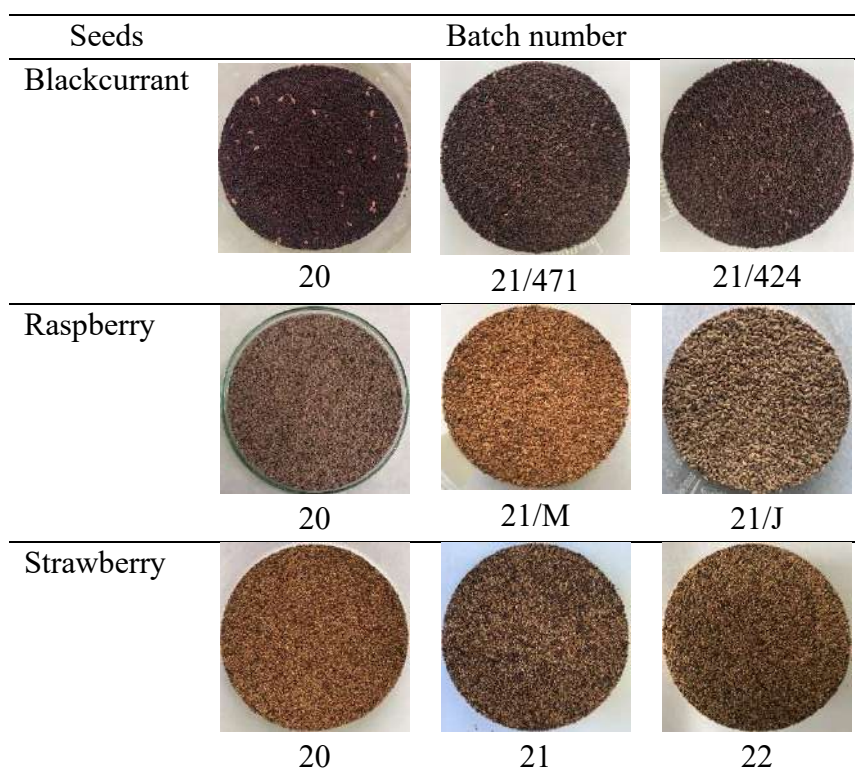


Figure 2. Blackcurrant, raspberry, and strawberry seeds from different production batches

3.2 Methods

3.2.1 Berry seed oil extraction and pre-processing

Berry seeds were cold-pressed using a kitchen-scale oil presser (Counter Intelligence Oilpresso, Netherlands) at 40 °C by default. The resulting oils were then cold filtered to eliminate the seed debris. In the preliminary optimisation experiments (P1), oils with and without seed debris were prepared. Oils with seed debris were only cold filtered after being cold pressed, they contained less than 5% (w/w) of seeds debris. Meanwhile, oils without seed debris were centrifugated (MPW Med. Instruments, Warsaw, Poland) at 978× g (5000 rpm) for 10 min after cold filtration.

To ease up n-hexane and supercritical CO₂ extraction process, berry seeds were crushed into fine powder before the extraction. During n-hexane extraction, 100 g of ground berry seeds were mixed with n-hexane in a 1:2 (w/v) ratio (Sigma-Aldrich, Steinheim, Germany). After 30 min of continuous stirring, the mixture was moved to an evaporation flask. A rotating vacuum

evaporator (The Rotavapor® R-300, BÜCHI Labortechnik AG, Flawil, Switzerland) was used to evaporate hexane under the following parameters: water bath temperature of 50 °C, 30 rpm, with 264 mbar of internal pressure for 30 min. Once all the hexane had been evaporated, the instrument was stopped. To eliminate the leftover hexane, the resulting oils were poured into a glass vial and purged with nitrogen gas for $\pm$ 10 min.

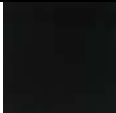
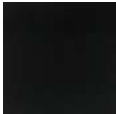
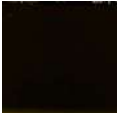
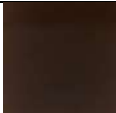
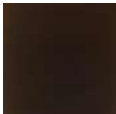
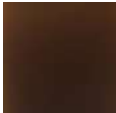
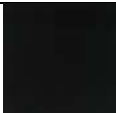
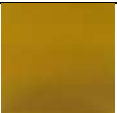
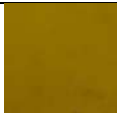
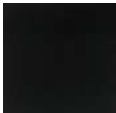
Berry seed oils	Batch	Cold pressing	Hexane extraction	Supercritical CO ₂ extraction
Blackcurrant	20			
	21/471			
	21/424			
Raspberry	20			
	21/M			
	21/J			
Strawberry	20			
	21			
	22			

Figure 3. The appearance of berry seed oils from cold-pressing, hexane, and supercritical CO₂ extraction.

In supercritical CO₂ extraction, ground berry seeds ($\pm$ 20 g) were filled into the extraction vessel. A multi-vessel supercritical extraction system (MV-10ASFE Waters, Manchester, MA, USA) comprising a CO₂ cylinder, cooling system, fluid delivery module, column oven, back pressure regulator, heat exchanger, fraction collection module, and ChromScope v1.20 software (Waters, Manchester, MA, USA), was used to perform the

extraction. The extraction parameters were adjusted to 325 bar of pressure, 40 °C for the oven, and 0.2 ml/min for make-up flow. The durations for dynamic 1, static, and dynamic 2 were: 10, 30, and 110 min, respectively. The oil that was produced was gathered in a glass container that was connected to the extractor device.

After each extraction, the resulting oils were directly kept at -80 °C in the brown-glass bottle with headspace filled with nitrogen gas (99.99%) to minimise quality deterioration.

3.2.2 Storage experiment

Three batches of berry seed oils (blackcurrant, raspberry, strawberry) were used in storage experiment. The storage study covered a period from fresh oil to 12 months of shelf life. At each storage stage (0, 3, 6, 9, and 12 months), analyses were performed using freshly opened bottles of each oil sample. Samples were stored in air-tight conditions at room temperature (± 20 °C), close to a window to allow exposure to natural sunlight. This was intended to simulate real-life conditions that oils might be subjected to during transportation, retail storage, or typical consumer use. Over the course of the storage observation, a total of 45 oil samples (15 samples from each type of oil) were examined.

3.2.3 Physicochemical analysis

The acid value (AV) was calculated in accordance with ISO 660 (2009). The amount of KOH needed to neutralise free fatty acids in 1 g of fat was indicated by the obtained value, which was given as mg KOH/g. The peroxide value (PV), which was measured in milliequivalents of active oxygen per kilogram of oil (mEq O₂/kg oil), was performed following the method described in ISO 27107 (2008) and p-anisidine values (p-AnV) were calculated using the procedure stated in ISO 6885 (2016). Oil's specific absorbance coefficients (K-values) were examined using the methodology described in AOCS Ch 5-91 (2009). The content of conjugated dienes and trienes were expressed in absorbance unit and represented as K₂₃₂ and K₂₆₈ values, respectively. The DPPH radical scavenging activity was measured in μ mol of Trolox equivalent (TE) per gram of oil following the methodology specified by Sanchez-Moreno *et al.* (1998).

Colour analyses were conducted using Konica Minolta CM-5 spectrophotometer (Konica Minolta, Inc., Tokyo, Japan). The CIELAB colour system (L*, a*, b*) was used to examine the colour characteristics of berry seed oils. The results were also expressed as chroma (C*_{ab}) and angle hue (h_{ab}). Angle hue depicts the colour wheel's gradation, whereas chroma shows the relative saturation. In storage study, the colour differences between every

observation were also presented as ΔE . These parameters were calculated using the following equations:

$$C_{ab}^* = \sqrt{(a^{*2} + b^{*2})} \quad (1)$$

$$h_{ab} = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (2)$$

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (3)$$

3.3.2 DSC thermal analysis

The melting and crystallization profiles of berry seed oils were determined using the methodology outlined by Tomaszewska-Gras (2016) with a small adjustment. A PerkinElmer differential scanning calorimeter DSC 8500 (Waltham, Massachusetts, USA) coupled with Intracooler II and Pyris software was used in the investigation.

The instrument was adjusted according to the following programs to analyse the phase transitions of berry seed oils: 1) heating at 30 °C, 5 min; 2) cooling from 30 °C to -67 °C at 1 or 2 °C/min; 3) heating from -67 °C to 30 °C at 1, 2, or 5 °C/min. From the melting and crystallization curves, DSC parameters such as peak temperature (T), peak height (h), and enthalpy (ΔH) were obtained (Fig 4). Every measurement was conducted twice for each sample.

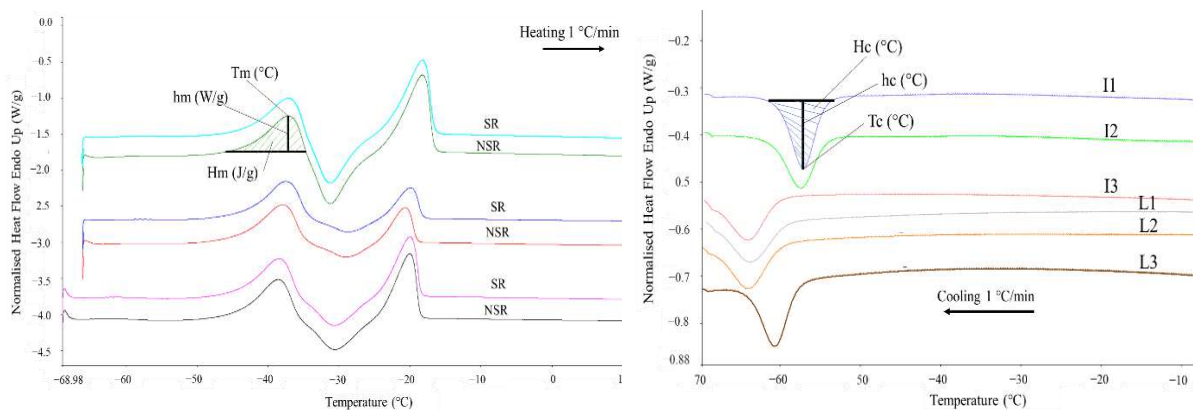


Figure 4. DSC parameters from melting (left) and crystallization (right) curves of raspberry seed oils

Thermo-oxidative stability of berry seed oils was studied based on isothermal and non-isothermal methods using a PerkinElmer DSC 7. During isothermal analysis, the oxidative stability of berry seed oils was analysed in accordance with the following program: 1) heating from 25 °C to 120 °C or 140 °C at 20 °C/min under nitrogen atmosphere; 2) isotherm at 120 °C or 140 °C for 2 min, convert the nitrogen to oxygen flow; 3) isotherm at 120 °C for 180 min or at 140 °C for 90 min. From the normalised heat flow in isothermal analysis, several oxidative stability parameters namely oxidation induction time (OIT, min), oxidation end time (OET, min), length of oxidation (Δt , min), heat flow (Y, W/g), and oxidation rate were obtained. Oxidation rate was calculated as follows:

$$\text{oxidation rate} = \frac{(Y1-Y2)}{\Delta t} \quad (4)$$

The first heat flow (Y1) was obtained at the same point as OIT, whereas the second heat flow (Y2) was at OET. The length of oxidation was obtained by subtracting OET to OIT.

Measurements were conducted in duplicate.

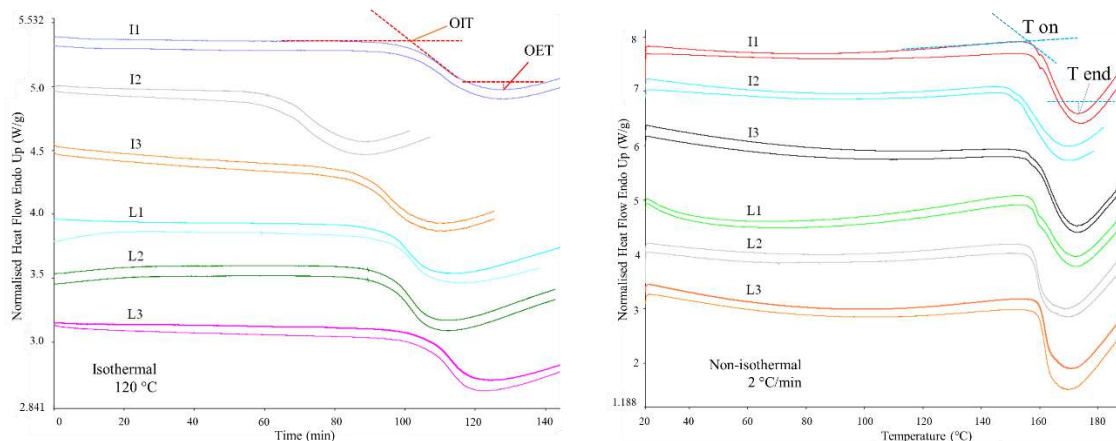


Figure 5. DSC parameters from isothermal (left) and non-isothermal (right) curves of raspberry seed oil

In non-isothermal analysis, the instrument was adjusted to the following program: 1) isotherm at 25 °C for 1 min; 2) heating from 25 °C to 200 °C at 2 or 5 °C/min under 20 mL/min of oxygen flow. The oxidation onset temperature (T_{on}) and the end temperature (T_{end}) were determined from the oxidation curve acquired during non-isothermal analysis. T_{on} was the intersection of the tangent line to the descending exotherm and the extrapolated baseline. The point at which the decreasing exotherm achieves the minimum of normalized heat flow was addressed as the T_{end} .

3.3.3 Compositional analysis

Analysis of fatty acid composition was carried out based on AOCS Ce 1h-05 (2005) method. The analysis was performed using Trace 1300 with a FID detector (Thermo Scientific, Waltham, MA, USA) and a SP TM-2650 capillary column (100 m × 0.25 mm × 0.2 μm) (Supelco, Bellefonte, PA, USA). Methyl heptadecanoate (Sigma Aldrich, USA) was used as an internal standard for the quantification of fatty acid content. The results from fatty acid composition were reported in mg/g oil.

Determination of tocopherol content was conducted using a Waters HPLC system (Waters, Milford, MA), which included a pump (Waters 600), fluorometric detector (Waters 474), photodiode array detector (Waters 2998 PDA), autosampler (Waters 2707), column oven (Waters Jetstream 2 Plus), and LiChrosorb Si 60 column (250 × 4.6 mm, 5 μm) by Merck (Darmstadt, Germany). To measure the amount of α-, β-, γ-, δ-tocopherols, and vitamin E in

the oil, the tocopherol content was assessed using the methods by Siger et al. (2014). The amount of tocopherol in the oil was stated as mg /100 g oil.

Volatile compounds analysis during storage of berry seed oil was carried out in non-targeted approach using flash gas chromatography (FGC) equipped with Heracles II E-nose (Alpha MOS, Toulouse, France) and two capillary columns (length 10 m, diameter 180 μ m) consist of polar (MXT-1701 14% cyanopropylphenyl/ 86% dimethyl polysiloxane) and non-polar fraction (MXT5: 5% diphenyl, 95% methylpolysiloxane). A 20 mL clear-glass vial was filled with berry seed oils (± 2 g), which were then sealed with a magnetic vial cap. Two replications of each analysis were performed. The vials remained in a shaker oven for 20 min at 40 °C and 500 rpm. From the vial's headspace, the volatile compounds were taken by syringe (70 °C) with Tenax TA® trap (40 °C, 60 s) as the adsorbent and a spitless injector (200 °C, injection speed 100 μ L/s, carrier gas flow at 30 mL/min). Subsequently, volatile compounds were desorbed from trap (240 °C, 93 s) and injected into two separated columns (40 kPa, 5 mL/min). The following thermal program was used to perform FGC E-Nose analysis: 1) set the temperature to 40 °C and maintain for 2 s; 2) set it to 80 °C at 1 K/s; and 3) set it to 250 °C at 3 K/s. Hydrogen as a carrier gas was used under the pressure of 40 kPa to 64 kPa with the increasing rate of 0.2 kPa/s. Flame ionisation detector (FID) operating at 260 °C was utilised to detect the separated volatile compounds. Signals were digitalised every 0.01 s and whole process of data acquisition was recorded in Alphasoft ver. 14.5.

Using the method specified by Romanić et al. (2021), the total carotenoid content was calculated and expressed in mg/kg of oil. The amount of chlorophyll was measured using AOCS cc13i-96 (2009b) and reported as mg of pheophytin A/kg of oil.

3.3.4 Spectroscopy analysis

Prior to UV/Vis spectra collection, berry seed oils were filtered through filter paper to remove physical impurities. The filtered oil was then added to pure isooctane (2,2,4-trimethylpentane) solution at ratio of 1:1 and mixed thoroughly. The oil's absorptivity was conducted following the AOCS Ch-591 (2009a) method at the range of 200 to 750 nm. Spectra collection was conducted using a Cary 1E spectrophotometer (Varian, Belrose, Australia) using a quartz cuvette. The measurement was conducted in triplicate.

FTIR spectra of berry seed oil were collected at the range of 4000 to 500 cm^{-1} (32 scans/sample or background) at 4 cm^{-1} resolution in reflectance mode. The spectra were collected using a Spectrum Two FT-IR spectrometer with Universal ATR and a diamond crystal (PerkinElmer, Waltham MA, USA). By using the diamond crystal surface as the

background, oil ($\pm 50 \mu\text{L}$) was dropped onto the top of the surface and evenly spread. The spectra were collected in duplicate.

3.3.5 Data analysis

A Tibco Statistica 13.3 software (Tibco Software Inc., Tulsa, OK, USA) was used to conduct all statistical tests with a significant level of 5%. Before determining statistical differences, the data size and amount of group in a dataset should be stated and a suitable normality and homogeneity tests were performed. Shapiro-Wilk test was performed to check the normality, then followed by homogeneity tests such as Bartlett test or Levene's test. To determine differences between the means of three or more groups, an ANOVA test with Tukey's HSD as a post-hoc test was conducted. To quantify the linear relationship between two continuous variables, a Pearson's correlation analysis was performed. Additionally, to visualize the data pattern in 2D or 3D, principal component analysis (PCA) was carried out.

In physicochemical characteristics study during storage (P2), a multiple linear regression (MLR) analysis was performed to identify the prominent variables affecting thermo-oxidative stability of berry seed oils. A forward stepwise regression was employed to reduce non-significant variables. At the end of analysis, the regression formula only includes the most significant variables affecting oxidation induction time measured at 120 °C and 140 °C. The regression models were evaluated based on the value of R^2 , indicating the goodness of fit.

In determination of storage time based on volatile profiles (P4), partial least squares regression (PLS-R) was performed. This study used two types of datasets in PLS-R models, which are full FGC E-nose chromatogram and the selected range with variable importance in projection (VIP) score greater than one. Correlation Optimised Warping (COW) algorithm was applied to the full chromatogram to make the dataset from the same berry types aligned. Subsequently, these datasets were pre-processed using Pareto-scaling and mean-centring. Validation of PLS model was conducted using Venetian blinds cross-validation (10 segments) and the optimal number of latent variables (LV) were chosen. The PLS-R models were evaluated based on determination coefficients (R^2), root mean square error (RMSE), and residual prediction deviation (RPD) during calibration or validation.

During the authenticity screening of commercial berry seed samples (P5), partial least square discriminant analysis (PLS-DA) models were developed. The models were separately developed for DSC melting curves and FTIR spectra. DSC curves were normalised and pre-processed using baseline correction (automatic weighted least square, order = 2). Whereas SNV and Pareto scaling were applied to FTIR spectra. After pre-processing, PCA and PLS-DA were

applied to the datasets. Models were validated using Venetian blinds cross-validation (10 segments) and the optimal LVs were chosen according to global minimum of root mean square in cross validation (RMSECV). To evaluate the classification performance, a confusion matrix with sensitivity, specificity, and F1 scores was used.

Soft independent modelling by class analogy (SIMCA) model was employed to classify berry types and extraction methods (P6). The model consisted of a fused data of DSC melting curves and FTIR spectra. Prior to data fusion, both datasets were pre-treated separately. DSC curves were normalised and pre-processed with baseline correction (automatic weighted least square, order = 2). FTIR spectra used standard normal variate (SNV), mean-centring, and smoothing (SavGol, filter width: 15, polynomial order: 0, derivative order: 0). Cross-validation was carried out based on k-fold Venetian blinds. All chemometric analyses were conducted using Solo + MIA v.9.5 software (Eigenvector Research, Manson, WA, USA). Visualisation of Pearson's correlation was conducted using OriginPro 2024 v.10.1 software (OriginLab Corporation, Northampton, MA, USA). A complete chemometric workflow in P6 was depicted in Fig 6.

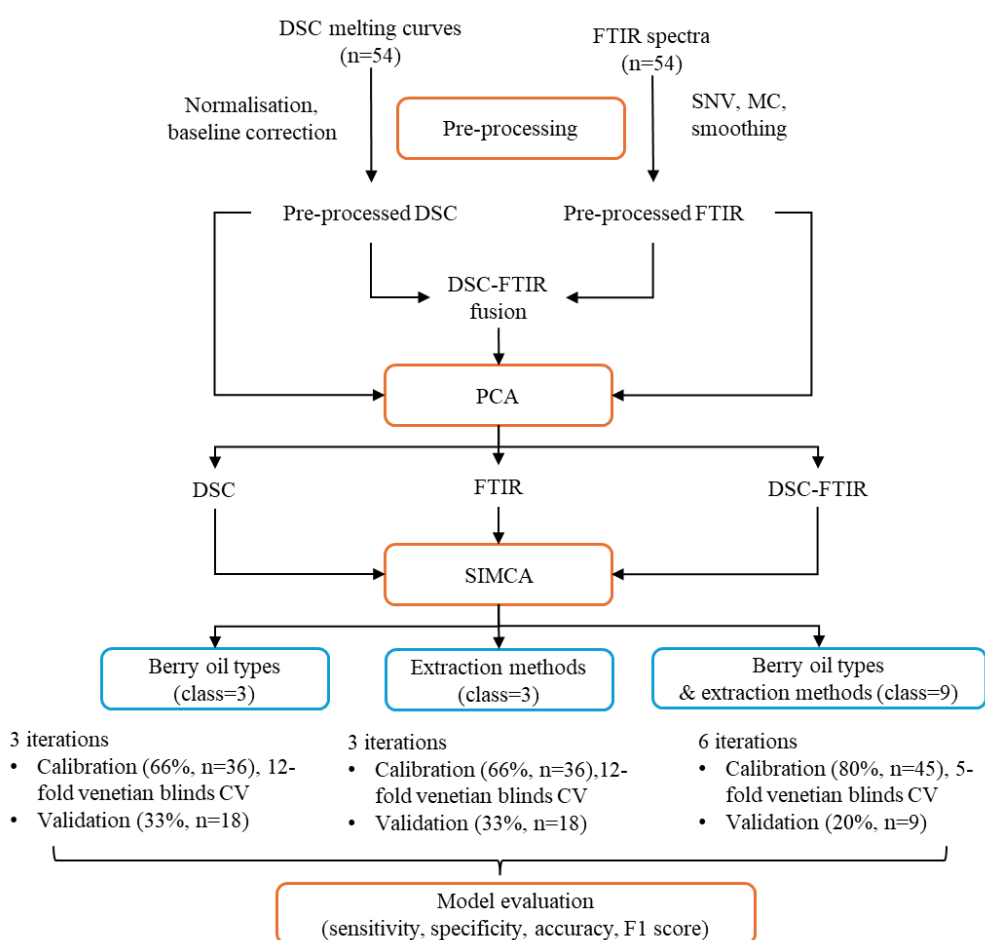


Figure 6. Chemometric workflow in paper 6

4. Research findings

4.1 Methods optimisation

This part of research aimed to verify the first hypothesis, whether seed freshness, clarification process, and analytical conditions for DSC and FTIR analysis affecting thermal and spectroscopic properties of berry seed oils. Additionally, this part aimed to establish an optimised thermal and spectroscopic methods for authenticity assessment of berry seed oils.

4.1.1 Influence of seeds quality and clarification process on the thermal properties of cold-pressed oil (P1)

Prior to the main study on the physicochemical characteristics of freshly pressed berry seed oils, a preliminary investigation of the influence of seeds' quality and clarification process on oil quality and thermal properties was conducted. The preliminary study employed three batches of raspberry seeds differing in months after expiration (0, 10, and 20 months). Expiration date was determined by the supplier, which is one year after the production date. Oils from these seeds were freshly cold pressed and directly filtrated to remove prominent debris. Afterwards, oils were divided into two groups, which were oils with seed residue (SR) and without seed residue (NSR). The oils labelled as NSR were subsequently processed into centrifugation step, in which seed residues were completely separated from the oil, resulting in clear and transparent appearance.

Quality characteristics of the oils were investigated based on the p-anisidine value (p-AnV) and DPPH radical scavenging activity. The oils from expired seeds possessed a higher p-AnV, indicating advanced stage of oxidation has occurred in seeds and this condition was naturally passed to the oil. The highest p-AnV was found in the oils extracted from the oldest seeds i.e. 20 months after expiration date. The storage time of seeds also influenced significantly the reduction of DPPH and thermo-oxidative parameters, especially OIT and oxidation rate ($p \leq 0.05$). When SR and NSR oils were compared, higher quality deterioration was found in NSR oils. It was connected to the DPPH radical scavenging activity, which was significantly higher in the oils with seed residues (around 7-9 times). These results, however, depend on the natural characteristics of the seeds. In the case of raspberry seeds, they are naturally rich in bioactive compounds namely ellagic acid, cynaidin-3-o-rutinoside, as well as quercetin (Marić et al., 2020). Following the presence of bioactive compounds, raspberry seeds residues in oil were beneficial as they helped to protect the oil against oxidative deterioration.

In practice, cold filtration was conducted to remove prominent debris of seed residue directly after obtaining the crude oil from cold pressing. This process improved the visual

appearance and oil stability at the first stage of production and prevented the unwanted sedimentation. However, a complete removal of seeds residue by centrifugation or advanced separation process were shown to negatively impact the oil quality. It can be concluded that a complete removal of seed residue was not necessary to be conducted. This study also highlighted that initial seeds quality is strongly correlated with the resulting oils. As a by-product of berry processing, berry seeds might need a longer processing time compared to other oilseed raw material. The seeds must be dried, sieved away from pomace, and frozen to keep the freshness, and thawed prior to extraction.

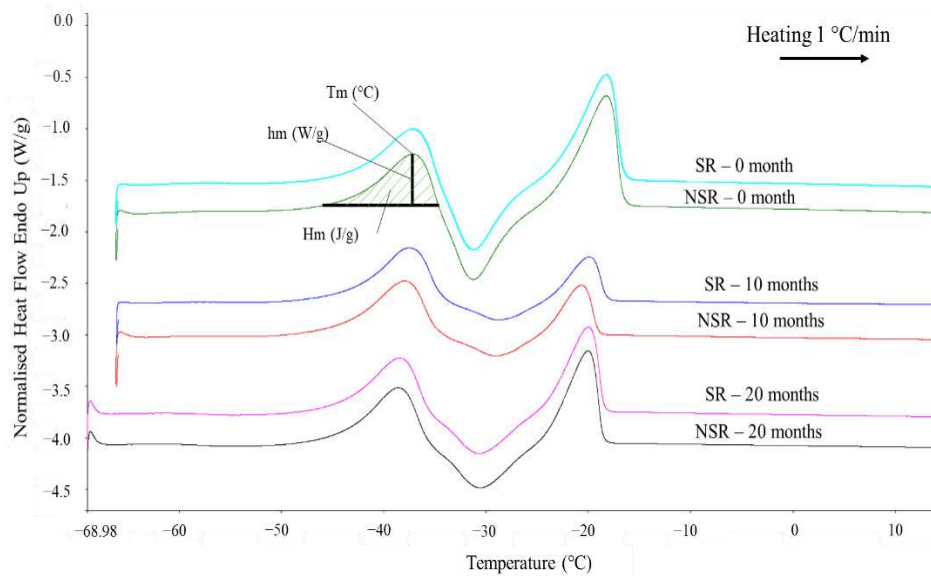


Figure 7. Thermal properties of berry seed oils differing in seed's age (0, 10, 20 month) with seed residue (SR) and without seed residue (NSR)

In terms of thermal profile, the presence of seeds' residue in oil did not influence the shape of melting and crystallization phase transition profiles of raspberry seed oil (Fig 7). This is because the shape of melting and crystallisation curves was influenced by fatty acid composition, which does not differ in both SR and NSR groups. Regarding the influence of seeds' age on phase transition profiles, differences in melting profiles were observed, which was expressed by significant differences in enthalpy values between oils from fresh seeds and expired seeds ($p \leq 0.05$). Contrary to phase transition profiles, raspberry seed oils from the SR and NSR groups differed in oxidative stability. The oil from the SR group and pressed from fresh seeds, exhibited the highest oxidative stability. This preliminary study concluded that the clarification step did not result in distinguishable phase transition profiles of oils but rather affected its oxidative stability. While seeds age affected both the phase transition profiles and the oxidative stability of the oils.

4.1.2 Investigation of factors affecting thermal and spectroscopic profiles (P2)

In the next stage, the aim was to investigate whether berry seed oils possessed distinguishable thermal and spectroscopic profiles, so they could be used as reference markers for authenticity studies. Prior to verifying the hypothesis, a preliminary study aimed at optimising thermal and spectroscopic methods was conducted. Thermal and spectroscopic profiles of raspberry seed oils were studied using oils extracted in laboratory and purchased from the industry (P2). Beside exploring the most suitable analytical parameters and condition for authenticity assessment, this study also aimed to investigate the natural variability of raspberry seed oils. Thermal profiles were analysed using DSC technique, while spectroscopic profiles were captured using UV/Vis and FTIR spectroscopy.

In DSC experiments, different scanning rates (1, 2, 5 °C/min) were tested to capture the melting and crystallization curves based on the analysis of raspberry seed oils. Lower scanning rates gave the highest peak resolution, however longer analysis time was needed to run one sample. Crystallisation curves at 1 °C/min presented a well-shaped exothermic peak compared to the same curves obtained at 2 °C/min. In the case of melting curves, higher peak resolution was obtained at the heating rate 1 and 2 °C/min but with a lower limit of detection. Regardless the peak resolution, all tested heating rates were able to distinguish samples that differed in fatty acid composition (P2; Fig 3 B, D, E). Additionally, DSC melting curves were more suitable for detecting differences between laboratory-pressed and commercial oils than crystallization profiles regardless of the scanning rates. Since melting curves obtained at heating rate 5 °C/min showed striking differences in peaks shape between groups, this rate was chosen to be the most suitable parameter for the further studies on the authenticity assessment.

The potential of spectroscopic techniques to differentiate between groups of raspberry seed oils was also evaluated. Distinctive properties of oils were captured in specific regions of UV/Vis and FTIR spectra (Table 1). Different concentrations of specific compounds were indicated by different intensity in each designated region. These spectroscopic profiles do not only capture the presence of aromatic and organic compounds, but also the quality parameters. Along with DSC melting curves at 5 °C/min, spectroscopic profiles were valuable to be used during rapid quality and authenticity assessment of raspberry seed oils.

Table 1. Distinctive UV/Vis and FTIR spectral range in berry seed oils

Instruments	Range	Compounds or functional groups
UV/Vis wavelength (nm)	216	π - π * transitions
	220 – 250	Carboxylic and phenolic acid moieties
	234, 270	Conjugated dienes, trienes
	260 – 340	Aromatic ring, tocopherols, phenolic acid (gallic, caffeic, ferulic)
	420, 443, 473	Carotenoids
	670	Chlorophyll
FTIR wavenumber (cm ⁻¹)	721	<i>Cis</i> double bonds
	950 – 988	C-H <i>trans</i> - and <i>cis</i> - conjugated diene of hydroperoxide
	1046	-C-O
	1408	=C-H <i>cis</i> -
	1712	Free fatty acids
	1726	-C=O acids
	2931	-C-H(-CH ₂)
	3470	-OH hydroxyl group

In summary, the first stage of the research (P1, P2) has addressed the first hypothesis, demonstrating that optimised thermal and spectroscopic methods can provide reliable analytical parameters (heating rate, spectral range) and processing conditions for the authenticity assessment of berry seed oils.

4.2 Physicochemical characteristics of authentic berry seed oils (P3)

At the second stage of research, physicochemical characteristics of three cold-pressed berry seed oils (blackcurrant, raspberry, strawberry) were determined following the fatty acid composition, tocopherols, total carotenoids, chlorophyll, colour properties (chroma, angle hue), and oxidative induction time (OIT). The study also included the storage experiment that covered a period from fresh oil to 12 months of shelf life. At each observation month (0, 3, 6, 9, and 12 months), physicochemical analyses were performed, and average values are presented in Table 2.

Three types of cold-pressed berry seed oils were shown to have distinctive physicochemical properties. Blackcurrant seed oils were distinguishable because of the higher content of carotenoids, chlorophylls and γ -linolenic acid (c18:3, *n*-6); raspberry seed oils had the highest antioxidant activity (DPPH), tocopherols content and thermo-oxidative stability (OIT), while strawberry seed oils contained the highest amount of α -linolenic acid (c18:3, *n*-3) and possessed distinctive colour characteristics (P3, Fig 3A).

Table 2. Physicochemical characteristics of cold-pressed berry seed oils

Parameter measured	Blackcurrant	Strawberry	Raspberry
Fatty acid composition (mg/g)			
c16:0	38.40	32.54	26.74
c18:0	14.95	12.07	12.02
c18:1	165.17	131.08	116.02
c18:2	320.97	360.62	431.10
c18:3 <i>n-6</i>	37.96	1.15	0.14
c18:3 <i>n-3</i>	37.30	174.20	129.72
Total tocopherol (mg/100 g)	97.28	54.09	173.00
Total carotenoid (mg/kg)	8.08	2.46	4.12
Chlorophyll (mg pheophytin A/kg oil)	18.96	8.25	11.20
Chroma (unit)	43.64	73.81	98.35
Angle Hue (°)	75.09	80.92	80.55
OIT 120 °C (min)	71.01	33.82	82.95
OIT 140 °C (min)	16.98	7.65	21.19

Physicochemical changes of berry seed oils during one year of storage were also investigated. Berry seed oils contained a high level of tocopherols; it ranged from 54 mg to 173 mg/100 g of oil. This property makes the oils remained stable after one year of storage, despite minor reductions in antioxidant activity as well as the increment of some deterioration indicators (AV, p-AnV, K-values). This stability was confirmed by OIT values measured at 120 and 140 °C by DSC technique, which remained stable throughout the storage. The thermo-oxidative stability was maintained due to abundant presence of tocopherols and carotenoids in samples, which only decreased up to 25% for tocopherols and up to 16% for carotenoids in all three oils, respectively. A multiple regression analysis revealed that during one year of storage, some factors namely tocopherol, carotenoids, and of oleic acid (c18:1) content were positively correlated with thermo-oxidative stability. In contrast, the stability was negatively affected by chlorophyll and conjugated trienes (K₂₆₈). This finding highlighted the role of tocopherols as a natural antioxidant to prolong the product's shelf life. Prior to an optional refining step, producer should estimate the targeted amount of tocopherol in finished products, noting that the selling point of berry seed oil lies on its bioactive compound and antioxidative properties. In terms of visual appearance, pigment degradation (chlorophyll, carotenoids) of berry seed oils became noticeable after 6 months as indicated by colour parameters changes (angle hue, chroma, ΔE). As chroma value gradually increases, oil's colour became more vivid until the end of storage.

To verify the second hypothesis (H2), it can be concluded that authentic berry seed oils possessed distinctive physicochemical properties that can be used as reference markers for

authenticity assessment. These characteristics have not changed significantly throughout storage. The oils were highly stable, and only minor changes were observed after one year.

4.3 Authenticity assessment of berry seed oils

The third research stage focused on the authenticity assessment of berry seed oils samples differing in storage times, extraction methods, and botanical origin from commercial samples. Hence, it aimed to answer the three remaining hypotheses, whether berry seed oils are distinguishable based on storage time (H3), as well as investigating the feasibility of DSC and FTIR techniques for rapid authentication of berry seed oils (H4) and their discrimination according to different extraction methods (H5).

4.3.1 Storage time determination (P4)

During preliminary study, melting profiles of berry seed oils throughout storage were also investigated (Fig 8). Melting profiles of both blackcurrant and strawberry seed oils remains in similar shape regardless of storage time. In contrast, differences in peak heights of exothermic (± 29 °C) and endothermic (± 21 °C) peaks from raspberry seed oils were observed during storage. As the composition of fatty acid remains stable after the storage experiments (P3, Fig 2A), polymorphic changes in raspberry seed oils might be caused by quality deterioration. It is confirmed by strong correlations between peak heights (hm) and quality indicators (p-AnV, K232, K268, AV) as depicted in Fig 9. Similar findings related to the lowering peak heights during storage observation were also reported in flaxseed oils after 6 months (Islam et al., 2023).

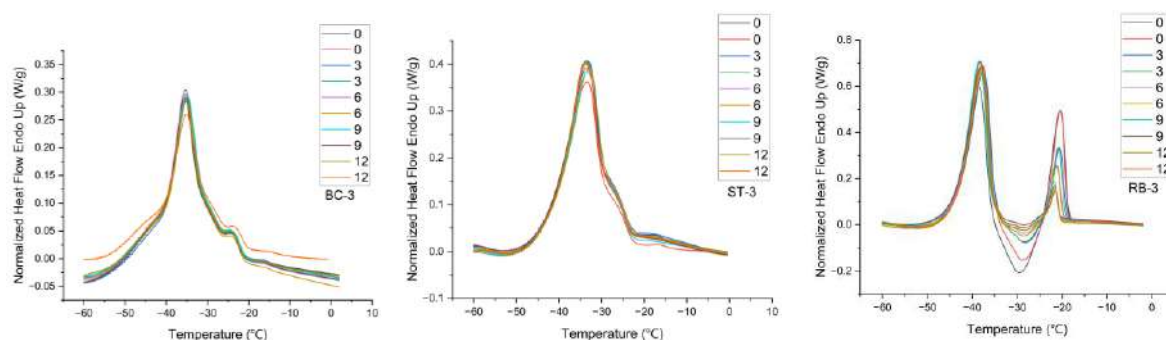


Figure 8. DSC melting profiles of blackcurrant (left), strawberry (middle), and raspberry (right) seed oils during storage

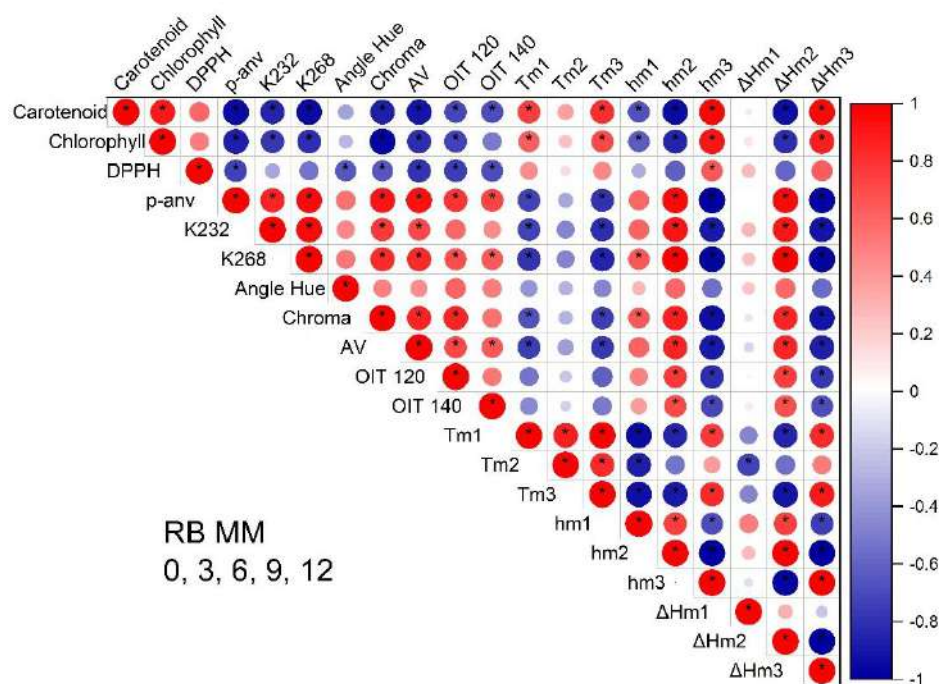


Figure 9. Pearson's correlation of DSC melting parameters in raspberry seed oils during storage

Following the minor changes in physicochemical and thermal properties, it was not possible to determine the storage time of berry seed oils based on the results obtained in previous stage. Hence, an additional assessment based on volatile profiles using Flash GC E-nose was conducted on the same oils. Principal component analysis (PCA) visualised a clear distribution according to berry types, suppliers, and storage times (P4, Fig 2). According to X-loadings score, factors influencing the sample distribution can be investigated further. In the function of storage time, the distribution was influenced by different volatile intensities observed at the retention time of 2400, 3200, and 4100. Peaks at 3200 and 4100 were identified as hexanal and 2-hexenal, which represent the oxidation of linoleic and linolenic acid, respectively (Melucci et al., 2016; Xu et al., 2018). Whereas peak at 2400 was recognised as 1-penten-3-ol, which indicates decomposition of hydroperoxides from *n-3* fatty acids.

To estimate the storage times, partial least square (PLS) models were developed for each berry oil types. Initial PLS models employed a full chromatogram from the retention time of 1800 to 8000 (10^{-2} s), this original dataset contains 6200 variables. These models were built based on relatively high amounts of latent variables (9.5 ± 1.5), making its unstable and in high risk of overfitting. The models' performance was then improved by reducing the least contributing variables as assessed by VIP score ($VIP < 1$). The second PLS models employed only 905 to 1535 variables with less latent variables (5.2 ± 1.1). Selecting specific chromatogram regions significantly improved the residual prediction deviation (RPD) from 2.8

to 5.7, indicating the suitability of the developed models to predict storage time of berry seed oil during quality control.

Considering the third hypothesis (H3), this study concluded that differentiation based on storage time was possible to conduct by assessing the volatile profiles of each berry seed oil. A rapid and reliable prediction of storage time was carried out using the developed chemometrics model.

4.3.2 Authenticity screening of commercial berry seed oils (P5)

Authenticity screening of commercial cold-pressed berry seed oils was conducted based on the established reference markers on physicochemical, thermal, and spectroscopic profiles presented in chapter 4.1 and 4.2. This study also investigated the feasibility of DSC and FTIR to rapidly evaluate the sample's authenticity compared to conventional analyses. Three types of berry seed oils (blackcurrant, raspberry, strawberry) were used in this study. The dataset was divided into testing and training set, which consisted of 68 lab-pressed oils and 38 commercial oils, respectively.

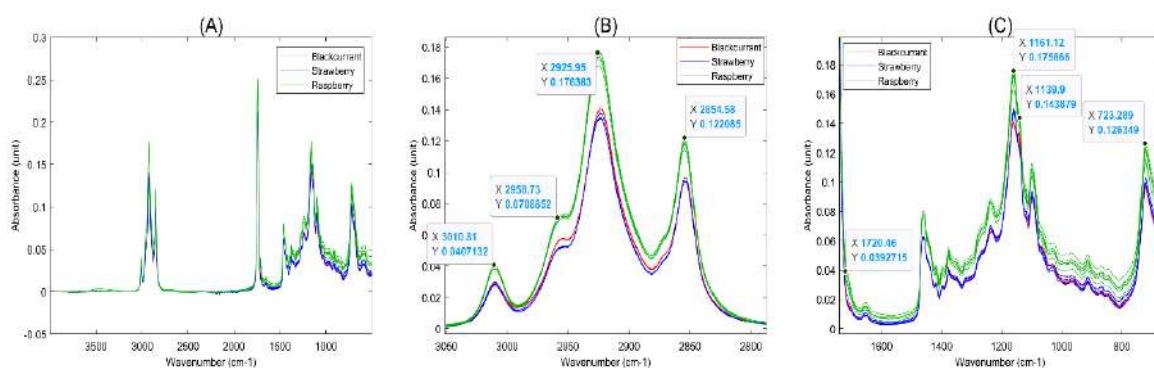


Figure 10. Raw FTIR spectra of berry seed oils (P5)

Previous studies by DSC utilised individual parameters such as peak temperature, peak height, and enthalpy, during authenticity assessment (Sanahuja et al., 2011; Tomaszewska-Gras, 2016b). In this stage of research new approach was proposed including a faster authentication method by coupling full DSC melting curves and FTIR spectra with chemometrics model to rapidly classify the oils samples. When PCA was performed on melting curves from training sets, a clear separation between berry types was observed (P5, Fig 3). In raw FTIR spectra, such separation was not clearly defined, indicating the needs to reduce the noise to maximise differences between berry types as it is shown in Figure 10.

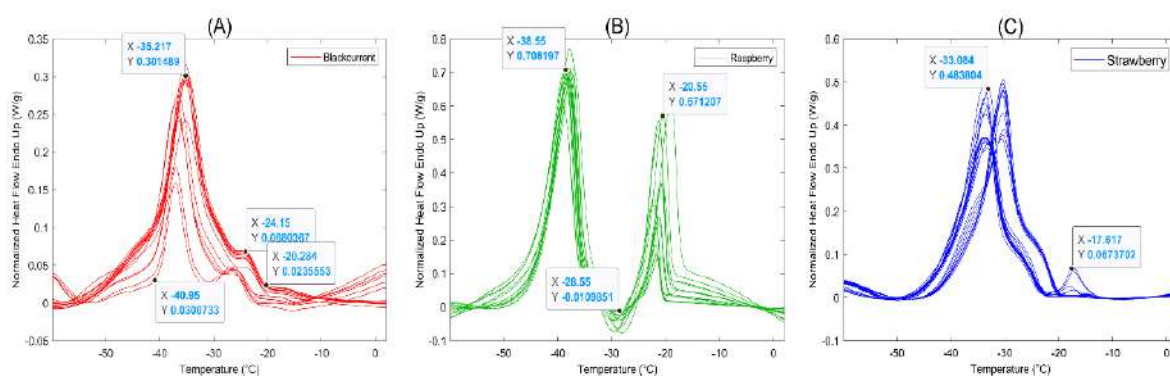


Figure 11. Berry seed oils with its distinctive DSC melting profiles (P5)

Compared to authentic DSC profiles of three berry seed oils, shown in Figure 11, totally different shapes of DSC curves were observed in commercial oil samples. The raw DSC curves and FTIR spectra of commercial berry seed oils are depicted in Figure 12. Considering the great variability in DSC curves, partial least square discrimination analysis (PLS-DA) with outlier detection was conducted to assign unclassified samples into a separate new class instead of forcing it to fit the pre-designed group. A personalised data pre-processing was applied to each DSC and FTIR dataset, subsequently VIP selection was also conducted. With supervised learning and proper data processing, classification performance by DSC and FTIR training models were improved as indicated by lower RMSECV and higher R^2 CV berry types.

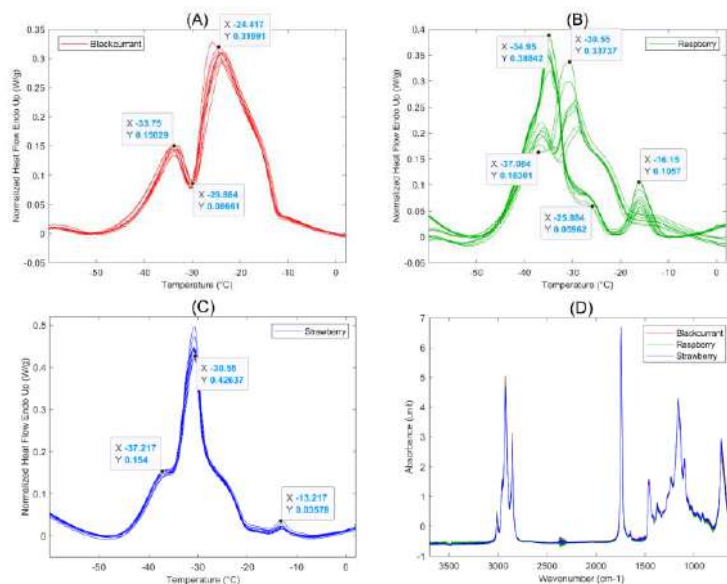


Figure 12. DSC melting curves and FTIR spectra of commercial berry seed oils (P5)

The developed models were then applied to testing data, with the goal to classify the berry types in commercial oils. DSC model demonstrated a good performance in recognising the melting curves pattern of berry seed oils and assigned it to the most similar-looking group. The model did not classify all commercial raspberry oils to the pre-defined class due to striking

differences in melting shape. The shape of DSC curves are dependent on the composition of fatty acid, which differs due to the seed varieties (Ispiryan et al., 2023) or possibly by adulteration. In models by FTIR, most commercial oils were classified to pre-defined class, with some samples assigned to unclassified class. Despite the excellent performances, there are some limitations that should be overcome such as using more complex training data that could cover natural variability of oils from different seed varieties, oil freshness, and different methods of extractions. If the training data is improved, further clustering analysis could be performed to determine the identity of unassigned samples.

Regardless of limitations, classification models based on DSC and FTIR performed well and offer room for improvement in solving more complex authenticity cases. Compared to physicochemical analysis, utilising both instruments during routine assessments could save a great amount of time. Hence, this stage of research has verified the fourth hypothesis (H4) regarding the feasibility of thermal and spectroscopic methods for authenticity assessment .

4.2.3 Discrimination of berry seed oils based on extraction methods (P6)

Following the feasibility study of DSC and FTIR during rapid authenticity screening, these techniques were then used to classify berry seed oils differing in extraction methods (cold pressing, n-hexane, supercritical CO₂ extraction). This aspect was important due to label transparency, considering the growing concerns of n-hexane usage in edible oil, which is not required to be declared in label following the existing EU law. Thus the last stage of research aimed to evaluate the DSC melting profiles and FTIR spectra according to the different extraction methods. In this experiment the innovative analytical approach has been applied i.e. data fusion of DSC and FTIR dataset to differentiate oils based on various extraction methods. It was stated that physicochemical properties of the oils were influenced by extraction methods, while fatty acid composition remains similar regardless of the extractions. According to the thermal profiles, the shape of DSC melting curves remains similar although slight shifting towards higher temperatures was observed in some endothermic peaks. Following this attribute, the group distribution from DSC profiles was only limited to berry types as shown in PCA, shown in Figure 13. Pearson's correlation showed that DSC peak temperatures are strongly correlated with parameters such as antioxidant activity, oxidative stability, tocopherols-, carotenoids- and fatty acid composition

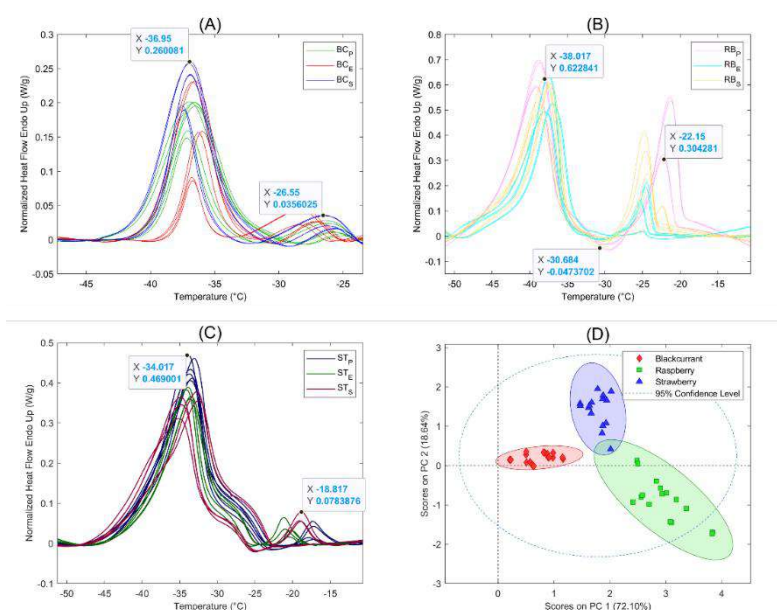


Figure 13. DSC melting curves and PCA of berry seed oils from different extraction methods

Contrary to the DSC melting curves, the FTIR spectra demonstrated a decent capability to emphasise differences between extraction methods but limited capability to differentiate between the berry types as shown in Figure 14. Some FTIR variables that highly contributed to the group distribution were wavenumbers at 2931, 1726, 1408, 1046 cm^{-1} . These FTIR variables possessed strong correlation with oil quality parameters and bioactive compounds. In respect DSC curves and FTIR spectra attributes to cover the sample's characteristics, both data were fused into one dataset called “DSC-FTIR” and the classification models were developed.

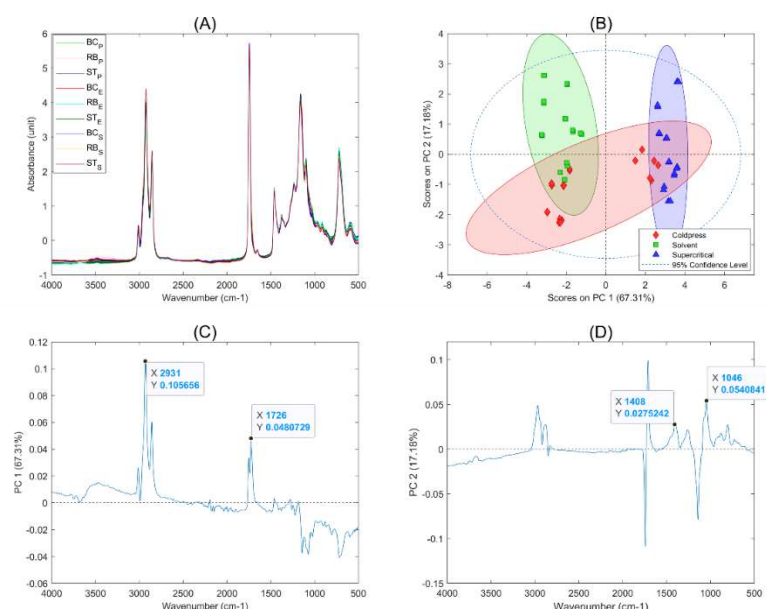


Figure 14. FTIR spectra and PCA of berry seed oils from different extraction methods

With a low-level data fusion and customised data processing, a clear distribution of berry types and methods of extraction were decently presented in PCA (Fig 15). From this

dataset, soft independent modelling by class analogy (SIMCA) algorithm was employed during classification analysis and the models built from DSC-FTIR, raw DSC, and raw FTIR datasets were compared. DSC-FTIR model outperformed the models from single analytical instruments, it emphasized the benefits of combining spectral data with thermal analysis to achieve more advanced purposes during oil analysis and food safety screening. Furthermore, this research approach significantly shortens the analysis time and costs to obtain information on compositional changes caused by different extraction methods. Regardless the performance and practicability, the model possessed limitations and could be improved by involving more samples during external validation.

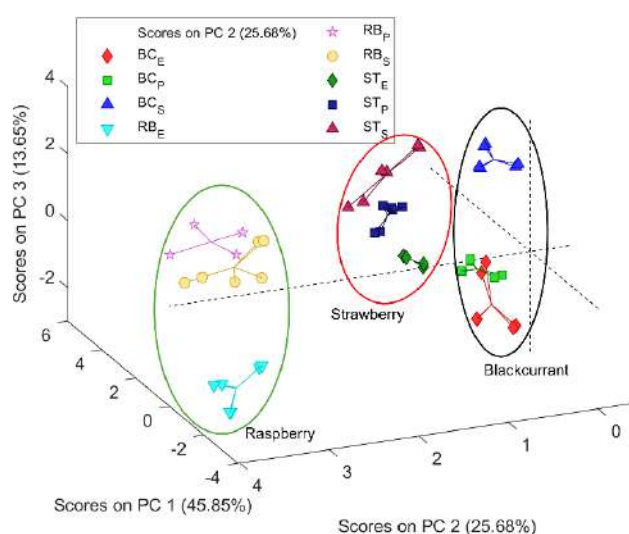


Figure 15. PCA of DSC-FTIR dataset (P6)

The results from this stage verified the last hypothesis (H5) regarding distinctive characteristics in thermal and spectroscopic profiles of berry seed oils from different extractions. Additionally, a good-performing classification model was achieved when both profiles (DSC and FTIR) were combined and optimised with proper chemometric techniques.

5. Novelty and limitation of the research

This work provided essential information about developing a non-targeted authenticity assessment specified for berry seed oils using thermal and spectroscopic techniques. The new approaches covered by this work are listed as follows:

1. Utilisation of full DSC curves and FTIR spectra

Previous advances using DSC for authenticity assessment utilised individual parameters such as peak temperature, enthalpy (peak area), and intensity of phase transition (peak height) from melting and/or crystallization curves. Instead of

measuring individual parameters, this work utilised full data from DSC curves and FTIR spectra and combined it with chemometrics. This approach improved the analytical efficiency by shortening the data analysis time, expanding the dataset and reducing error caused by subjective intervention during data mining.

2. Data fusion of full DSC profiles with FTIR spectra

This work introduced a low-level data fusion technique, combining thermal and spectroscopic data to detect berry seed oils extracted using n-hexane. It provides practical and efficient solution to solve the label transparency problem.

3. Comprehensive authenticity study on berry seed oils

Aside from the advances in analytical instruments and data processing, this work was the first attempt to study the comprehensive characteristics of authentic berry seed oils based on physicochemical, thermal, and spectroscopic data. The knowledge obtained from this study can be used as a base for food policy makers to build the regulatory standards for berry seed oils.

Regardless of the advances, a lot of improvements on chemometrics models can be implemented and direct the future studies, for example by developing model's robustness with seasonal variations and external validation using real case adulterants (e.g. rapeseed, sunflower oils). With a wider coverage of training data, better chemometrics models could be developed and more advanced purposes could be achieved. Summing up, this research work supports the circular economy implementation by expanding the knowledge about green technology and by-products valorisation to reach the long-term European Green Deal's goal.

6. Conclusions

Rapid and reliable methods for assessing the authenticity of berry seed oils based on physicochemical, thermal, and spectroscopic profiles were developed. Reference markers for authentic berry seed oils were identified, and a method was developed to address specific authentication challenges. This method coupled DSC melting curves and FTIR spectra with chemometrics, which significantly reduced the labour and time required for conventional analysis and improved the detection accuracy. Specific conclusions of each chapter are outlined as follows:

1. The quality and thermal properties of berry seed oils were influenced by the initial seed quality and the clarification process. Lower seed quality and the clarification process negatively affected oxidative stability and antioxidative properties. However, no

differences in DSC phase transition profiles were observed in relation to the clarification process.

2. The scanning rate affected the melting and crystallization DSC profiles of oils. Based on the results, the melting profile obtained at a scanning rate of 5 °C/min was considered the most useful for further authenticity studies of the oils.
3. Between the spectroscopic methods, FTIR was considered more informative than UV-VIS spectroscopy for further authenticity studies of the oils.
4. Each type of berry seed oil possessed distinctive physicochemical characteristics, which can be used as reference markers for authenticity assessment.
5. Berry seed oils remained stable in terms of the OIT index during one year of storage. In maintaining the thermo-oxidative stability of the oils at a constant level over this period, the most significant parameters influencing OIT values were the content of oleic acid (C18:1), tocopherols, and carotenoids, as revealed by the multiple linear regression analysis (MLR).
6. The determination of storage time was possible through the assessment of the volatile profile changes during storage using Flash GC E-nose and optimised PLSR models.
7. An advanced authentication method, employing the entire DSC and FTIR profiles instead of individual analytical parameters, was successfully developed by coupling the dataset with optimised chemometrics technique for the discrimination of berry seed oils.
8. During authenticity screening, the developed thermal and spectroscopic methods coupled with PLS-DA models accurately recognised the differences in melting curves and absorption intensity among commercial oils.
9. The SIMCA model, built from a fused dataset combining both DSC and FTIR profiles, outperformed models based on single analytical techniques in classifying oils according to berry types and extraction methods. This work suggests a new approach to utilising low-level data fusion for thermal and spectroscopic data to improve classification performance.

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Publications

Influence of Seeds' Age and Clarification of Cold-Pressed Raspberry (*Rubus idaeus* L.) Oil on the DSC Oxidative Stability and Phase Transition Profiles

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Abstract: After cold-pressing, small particles of seed residue remain in raspberry seed oil (RSO), even after passing it through cold filtration. The removal of the remaining seed residue is rather an alternative option to improve the visual properties of RSO. This study investigated the influence that the seeds' age (0, 10, 20 months) and clarification process after pressing has on the oxidative stability and phase transition of RSO by means of differential scanning calorimetry (DSC). The results proved that the oil centrifugation process reduces the DPPH radical scavenging activity and oxidative stability measured by p-anisidine value (p-AnV) and DSC oxidation induction time (OIT) at 120 °C of all RSO samples, regardless of the age of the seeds ($p \leq 0.05$). No significant differences were observed on the DSC melting and crystallization properties at 1 °C/min after the oil clarification by centrifugation ($p > 0.05$). The storage time of raspberry seeds, i.e., 10 and 20 months after expiry date, influenced the quality deterioration of RSO, as measured by higher p-AnV, lower DPPH, and OIT values ($p \leq 0.05$). The results presented provide new information about oil production processing, suggesting that producers should reconsider giving up the clarification process of oil, since it lowers all quality parameters.

Keywords: raspberry seed oil; antioxidant; oxidative stability; DSC; seed residue



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1. Introduction

Raspberry seed oil (RSO) has received attention in recent years due to its promising health benefits [1,2]. RSO is often extracted using cold pressing because the resulting oil is safe for direct usage or even for consumption (i.e., cosmetics, salad dressing, cooking oil). Depending on the moisture content and heating parameters, the resulting oil contains certain proportions of solid particles (i.e., 0.0058–0.0608% w/w) [3], which are often called mechanical impurities [4]. Visible mechanical impurities that impact oil turbidity are separated by precipitation or cold filtration from the crude oil. The separation of mechanical impurities in RSO was reported to produce a minor reduction in oxidative stability [5]. As the presence of the remaining seed residue may affect visual properties, smaller seed particles in filtered oils can be completely removed by centrifugation, if this is necessary [6]. The necessity to completely remove the seed particles might lead to ambiguation for the RSO producer, as the raspberry seed itself contains significant amounts of natural antioxidants.

Additionally, it is important for the RSO producer to understand the characteristics of oil obtained from different seeds' quality. Raspberry seeds are the by-product of raspberry fruit processing. Seeds obtained from by-product were mechanically damaged after fruit processing and needed to be processed further (i.e., sieving, drying) before oil extraction took place [7]. After the pre-treatment, the quality of raspberry seeds is influenced by the storage conditions (time, temperature) that may affect the hydrolytic and free-radical reactions in the seeds [8]. In view of the natural characteristics of seeds from by-product,

RSO, and high consumer demand, an urgent need to develop better processing parameters to produce high-quality RSO has emerged.

The use of differential scanning calorimetry (DSC) as rapid oxidative stability and authenticity instruments for cold-pressed oils has been reported [9]. DSC allows to determine the thermal properties of oils based on their fatty acid and triacylglycerol compositions without any need to use hazardous chemicals. Despite its feasibility, there is limited knowledge on using DSC to measure compositional changes in seed oil. Several studies have reported quality changes in oils relating to raw material quality [10], cold filtration [5], and seed residue removal [11]. To the best of our knowledge, there is still little information regarding the influence that removing residue has on the oxidative stability and phase transition of RSO by DSC. Hence, the aim of this study is to assess (a) the influence of seed residue removal and (b) raw material quality on the oxidative stability and phase transition of RSO by DSC. The study was conducted to expand the application of DSC for oil quality and authenticity assessment.

2. Materials and Methods

The samples consisted of six cold-pressed RSOs that differed in seed freshness and the presence of seed residue. Samples were cold-pressed from fresh seeds and expired seeds (10 and 20 months past their expiry dates, based on the label) as it is presented in Figure 1. Fresh and expired raspberry seeds were purchased from GreenField Sp. z o.o. (Warsaw, Poland). The seeds (300 g) were cold-pressed using oil presser (Counter Intelligence Oilpresso, The Netherlands) with a default outlet temperature at ± 45 °C [12], cold filtered, and directly kept at the brown-glass bottle. All of the seeds were only went through a single pressing step. The acid value of the resulting oils were 6.59 ± 0.03 , 10.06 ± 0.31 , and 5.17 ± 0.00 mg KOH/g fat for oils from expired (20 and 10 months) and fresh seeds, respectively. The resulting oils were then divided into two groups: oils with seed residue (SR) and without seed residue (NSR). Oils without seed residue were obtained by centrifugation (MPW Med. Instruments, Warsaw, Poland) at $978 \times g$ (5000 rpm) for 10 min. The seed residue content in the centrifuged oils was approx. 5% (*w/w*). All oils were stored -80 °C before analysis with the headspace filled with nitrogen gas (99.99%) to minimize quality deterioration.

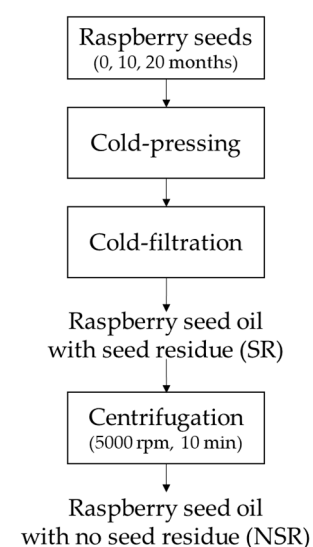


Figure 1. Flowchart to obtain raspberry seed oil (SR) and clarified without seed residue (NSR).

2.1. Chemical Analyses of Raspberry Seed Oil

2.1.1. p-Anisidine Value

Analysis of p-anisidine value (p-AnV) was conducted following the method described in ISO 6885 [13]. The value represents the number of secondary oxidation products that

react with the p-anisidine in acetic acid reagent. The absorbance reading was conducted at 350 nm.

2.1.2. DPPH Radical Scavenging Activity

RSO (300 mg) were weighed and diluted with 0.3 mL of ethyl acetate. The resulting sample solution (100 μ L) was mixed with DPPH radicals' solution (1 mM, 250 μ L) and 2 mL of ethyl acetate. After 20 min, the absorbance reading was conducted at 515 nm. DPPH value of RSO were expressed as μ mol of Trolox equivalent (TE) per gram of oil.

2.2. Thermal Analysis of Raspberry Seed Oil

Thermal analysis by DSC was performed based on the method described in ISO 11357-6 [14]. The oxidative stability and melting-crystallization profile of raspberry seed oil were analyzed.

2.2.1. Oxidative Stability Determination by Isothermal DSC

DSC isothermal analysis was performed at 120 and 140 $^{\circ}$ C, following the method reported by Islam et al. [15]. DSC isothermal analysis provided information regarding several oxidative stability parameters, such as oxidation induction time (OIT), oxidation end time (OET), length of oxidation (Δt), heat flow (Y), and the oxidation rate. The samples were analyzed in two replications.

2.2.2. Melting and Crystallization Profile by DSC

Melting and crystallization profiles were determined using the method described by Tomaszewska-Gras [16], with a slight modification. Melting curves were obtained by heating the sample from -70 to 30 $^{\circ}$ C at 1 $^{\circ}$ C/min. On the other hand, crystallization curves were obtained by cooling the sample from 30 to -70 $^{\circ}$ C at 1 $^{\circ}$ C/min. The measurement was conducted in two replications.

2.3. Statistical Analysis

Data analysis was conducted using Tibco Statistica 13.3 software (Tibco Software Inc., Tulsa, OK, USA). One-way ANOVA was carried out using Tukey's multiple range test ($\alpha = 0.05$).

3. Results and Discussion

3.1. Quality Characteristics of Cold-Pressed Raspberry Oil

Table 1 presents the quality characteristics of raspberry seed oils as measured by the p-anisidine value (p-AnV) and DPPH radical scavenging activity. The p-AnV expressed the amount of secondary oxidation product developed from peroxide decomposition. The analysis represented the advanced stage of oxidation that occurred in oils obtained from expired seeds. Oxidative stability measured by the p-AnV of unclarified oil with seed residue (SR) ranged from 4.56 to 11.53, whereas clarified with no-seed residue (NSR) oils ranged from 7.88 to 17.35. Generally, higher oxidative deterioration was observed in oils extracted from expired raspberry seeds. Significant differences in p-AnV among oils obtained from fresh (0 month) and expired raspberry seeds (10 and 20 months) were observed ($p \leq 0.05$). These results were in agreement with another study that reported the increment of the p-AnV after retesting the oil a year later than the expiry dates [17]. Besides seed freshness, the oxidative deterioration of the oils was also influenced by the absence of seed residue. Raspberry seed oils from the NSR group exhibited a p-AnV up to 42% higher than the SR group ($p \leq 0.05$). The results were in accordance with those of van Hoed et al. [5], who pointed out that filtering berry seed oil leads to a reduction in oil quality, as expressed by a higher p-AnV.

Table 1. Chemical characteristics of raspberry seed oil based on p-anisidine value (p-AnV) and DPPH radical scavenging activity.

Analysis	Months after Exp. Dates	SR	NSR
p-AnV	20	11.53 ± 0.52 ^{aB}	17.35 ± 0.09 ^{bC}
	10	10.51 ± 0.12 ^{aB}	13.27 ± 0.83 ^{bB}
	0	4.56 ± 0.61 ^{aA}	7.88 ± 0.12 ^{bA}
DPPH (µmol TE/g Oil)	20	190.74 ± 1.72 ^{bA}	18.96 ± 0.11 ^{aA}
	10	189.53 ± 3.01 ^{bA}	24.38 ± 0.81 ^{aB}
	0	206.87 ± 2.21 ^{bB}	30.88 ± 1.3 ^{aC}

SR: oil with seed residue, NSR: oil with no seed residue. Values are given as mean ± SD ($n = 3$). Different letters (a, b) in the same row indicate a significant difference ($p \leq 0.05$). Different letters (A, B, C) in the same column within the same analysis indicate a significant difference ($p \leq 0.05$).

Furthermore, the findings on the p-AnV were supported by the results from DPPH analysis. The SR group exhibited higher DPPH radical scavenging activity ($p \leq 0.05$), which resulted in a lower p-AnV than the NSR group. The results indicated the role of seed residue in protecting oil from oxidation. In contrast, another study reported that the presence of seed residue negatively impacted on the quality characteristics of cold-pressed sunflower oil [11]. It is worth noting that the influence of seed residue of oil depends on the natural characteristics of the seeds themselves. The ability of raspberry seed to protect lipid against oxidative damage was reported in another study [2]. The authors highlighted how the protective abilities of raspberry seed were influenced by the presence of bioactive component in seeds such as anthocyanin, namely cyanidin-3-o-rutinoside, as well as quercetin and other polyphenolic compounds. Additionally, raspberry seeds themselves were also considered a natural antioxidant in other studies, as they exhibited substantial amounts of ellagic acid, which was often linked to a significant antioxidative activity [1,18].

Seeds' age is shown to influence the gradual reduction of DPPH scavenging activity in the NSR group ($p \leq 0.05$). Older seeds might have been exposed to a longer peroxidation process that caused the depletion of natural antioxidant content in the resulting oil. Similar results regarding the reduction of DPPH value after storage of crude seed oil were reported by Chew et al. [19]. In contrast, no significant reduction in DPPH scavenging activity was observed between oils from expired seeds from the SR group (10 months and 20 months) ($p > 0.05$). Minor changes might be related to the protective ability from seed residue, as previously explained.

3.2. Influence of Seeds' Age and Oil Clarification on Oxidative Stability

The oxidative stability of raspberry seed oil was investigated based on DSC isothermal parameters at 120 and 140 °C, as presented in Table 2. Oxidative induction time (OIT) at 120 °C of SR group ranged between 96.01–106.42 min, which was significantly higher than the NSR group that ranged between 86.22–90.65 min ($p \leq 0.05$). The values of OIT in raspberry seed oil were in the same range as in previously reported studies [20]. In accordance with the p-AnV results (Table 1), oils with lower oxidative deterioration possessed a higher OIT value than oils extracted from older seeds. Significant differences in high OIT values were observed in 0-month oil from the SR group at 120 °C and in 0-month oils from both groups at 140 °C.

Table 2. Oxidative stability of raspberry seed oil based on DSC isothermal parameters (120 °C and 140 °C).

Isothermal (°C)	DSC Parameters	Months after Exp. Dates	SR	NSR
120	OIT (min)	20	96.51 ± 2.38 ^{bA}	86.24 ± 1.94 ^{aA}
		10	96.01 ± 2.44 ^{bA}	86.22 ± 1.11 ^{aA}
		0	106.42 ± 1.42 ^{bB}	90.65 ± 3.70 ^{aA}
	OET (min)	20	112.08 ± 1.22 ^{aA}	113.02 ± 0.57 ^{aA}
		10	108.63 ± 1.96 ^{aA}	115.5 ± 2.58 ^{aAB}
		0	122.01 ± 1.19 ^{aB}	122.02 ± 1.15 ^{aB}
	OIT-OET (min)	20	15.56 ± 1.16 ^{aA}	29.78 ± 2.52 ^{bA}
		10	12.62 ± 0.48 ^{aA}	29.28 ± 1.47 ^{bA}
		0	15.58 ± 2.61 ^{aA}	31.37 ± 2.54 ^{bA}
	Oxidation rate (W/g min)	20	0.003 ± 0.00 ^{aA}	0.014 ± 0.00 ^{bA}
		10	0.004 ± 0.00 ^{aA}	0.017 ± 0.00 ^{bA}
		0	0.003 ± 0.00 ^{aA}	0.012 ± 0.00 ^{bA}
140	OIT (min)	20	25.08 ± 0.8 ^{aAB}	25.80 ± 0.73 ^{aAB}
		10	23.43 ± 0.84 ^{abA}	22.83 ± 1.14 ^{aA}
		0	27.72 ± 0.90 ^{aC}	26.87 ± 0.93 ^{aC}
	OET (min)	20	35.56 ± 0.28 ^{aAB}	37.86 ± 0.63 ^{bA}
		10	33.24 ± 1.09 ^{aA}	36.09 ± 0.15 ^{aA}
		0	36.89 ± 0.51 ^{aC}	40.78 ± 0.87 ^{bB}
	OIT-OET (min)	20	10.48 ± 1.08 ^{aA}	12.05 ± 1.36 ^{aA}
		10	9.81 ± 0.25 ^{aA}	13.26 ± 1.30 ^{aA}
		0	9.18 ± 1.41 ^{aA}	13.91 ± 1.81 ^{aA}
	Oxidation rate (W/g min)	20	0.067 ± 0.00 ^{aA}	0.049 ± 0.01 ^{aA}
		10	0.073 ± 0.01 ^{bA}	0.043 ± 0.00 ^{aA}
		0	0.081 ± 0.01 ^{bA}	0.042 ± 0.00 ^{aA}

SR: oil not clarified, with seed residue, NSR: oil clarified, with no seed residue. Values are given as mean ± SD ($n = 2$). Different letters (a, b) in the same row indicate a significant difference ($p \leq 0.05$). Different letters (A, B, C) in the same column within the same analysis and the same DSC parameters indicate a significant difference ($p \leq 0.05$). OIT: oxidation induction time, OET: oxidation end time.

It is worth mentioning that despite the major differences in DPPH radical scavenging activity (Table 1) between the SR and NSR group, the OIT of the NSR group was only 10–16 min shorter. These results might indicate that DPPH radical scavenging activity does not significantly influence the OIT of oils. Oxidative stability in berry seed oil was linked to fatty acid composition, such as the content of polyunsaturated fatty acid and tocopherols [5]. Interestingly, differences in the OIT value between the SR and NSR groups were not observed at 140 °C ($p > 0.05$). The results indicated that the presence of seed residue does not have an impact on the oxidative stability of raspberry seed oil at higher temperatures i.e., 140 °C. It also indicates that a lower isothermal temperature, in this case 120 °C, is suitable for DSC oxidative stability assessment to differentiate oils that share similar general properties. These results show that the presence of substances with antioxidant activity from seed residue plays a significant role at the temperature of 120 °C, since the oxidation times (OIT) were very long, i.e., between 86.2 and 106.4 min., in contrast to the OIT test at 140 °C, where the time of oil resistance to oxidation was much shorter, between 22.8 and 27.7 min. Similar findings related to the ability of lower isothermal temperatures to differentiate oils from different seeds cultivar were reported in another study [15].

The length of oxidation in the SR group at 120 °C was shorter than in the NSR and ranged from 12.62–15.58 min ($p \leq 0.05$). Oxidation in the NSR group occurred at a higher oxidation rate than in the SR group at 120 °C ($p \leq 0.05$). The results indicate that raspberry seed oil from the NSR group became less stable than from the SR group, especially when

the oil was exposed to a certain temperature (120 °C) for a longer period of OIT (86–90 min). In contrast, the oxidative stability observation at 140 °C showed that the NSR group had a lower oxidation rate when the OIT value was shorter (25–26 min). Following these results, seed freshness was shown to have an insignificant influence on the length of oxidation and the oxidation rate of oil at any of the isothermal temperatures observed ($p > 0.05$). Oxidative stability findings from the study might constitute an important approach for the seed oil industry to design the processing parameters of RSO intended for cooking, especially if it includes heat exposure.

3.3. Influence of Seed Freshness and Oil Clarification on Melting and Crystallization Profile

The DSC melting profiles of raspberry seed oil obtained at a scanning rate 1 °C/min are presented in Figure 2. Raspberry seed oil from both groups possessed three melting peaks that consist of two endothermic peaks ($T_{m1} \cong -41$ °C, $T_{m3} \cong -22$ °C) and one exothermic peak ($T_{m2} \cong -39$ °C). Similar DSC profiles of raspberry seed oil were previously reported in other studies [21,22].

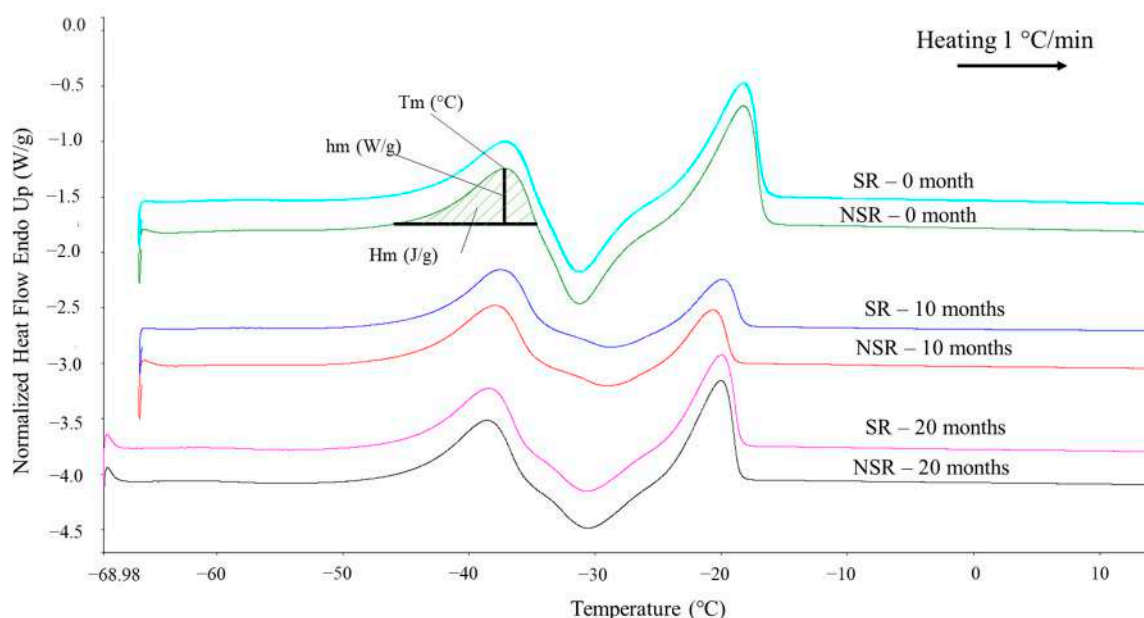


Figure 2. DSC melting curves of raspberry seed oil at scanning rate 1 °C/min. SR: oil not clarified, with seed residue, NSR: oil clarified, with no seed residue.

The results of DSC melting and crystallization analysis of raspberry seed oil at a scanning rate 1 °C/min are presented in Table 3. The presence of seed residue was shown to influence slight differences in the DSC melting and crystallization parameters. However, these differences were very small. In contrast to the statistical results, no specific differences in the shape of melting peaks were observed between the SR and NSR group (Figure 2). Thermal behaviors were influenced by the fatty acid composition of the sample [23]. According to Parry & Liu [18], the fatty acid composition of raspberry seed and its oil were similar to each other. Raspberry seed and its oil were reported to contain high amounts of oleic (C18:1), linoleic (C18:2 *n*-6), and α -linolenic (C18:3 *n*-3) fatty acid. Additionally, the authors (Parry & Liu [18]) highlighted that major differences between raspberry seeds and its oil were found in the higher amount of polyunsaturated fatty acid (PUFA) in the oil. This might explain the minor differences in thermal properties between both groups, as presented in Table 3.

Table 3. DSC melting and crystallization profile of raspberry seed oil at scanning rate 1 °C/min.

Melting	Months after Exp. Dates		SR	NSR
Peak temperature (°C)	Tm1	20	-41.02 ± 0.01 ^{aB}	-40.98 ± 0.23 ^{aA}
		10	-41.16 ± 0.19 ^{aAB}	-41.41 ± 0.04 ^{aA}
		0	-41.60 ± 0.01 ^{aA}	-41.42 ± 0.02 ^{bA}
	Tm2	20	-38.79 ± 0.04 ^{aA}	-39.07 ± 0.23 ^{aA}
		10	-38.92 ± 0.23 ^{aA}	-39.34 ± 0.05 ^{aA}
		0	-39.19 ± 0.01 ^{aA}	-39.08 ± 0.00 ^{bA}
	Tm3	20	-21.50 ± 0.08 ^{bA}	-21.94 ± 0.04 ^{aA}
		10	-21.76 ± 0.33 ^{aA}	-22.00 ± 0.01 ^{aA}
		0	-24.22 ± 4.89 ^{aA}	-21.77 ± 1.63 ^{aA}
Peak height (W/g)	hm1	20	0.07 ± 0.000 ^{aA}	0.08 ± 0.001 ^{bA}
		10	0.09 ± 0.003 ^{aB}	0.09 ± 0.000 ^{aB}
		0	0.07 ± 0.003 ^{aA}	0.08 ± 0.004 ^{aA}
	hm2	20	-0.26 ± 0.006 ^{bA}	-0.34 ± 0.004 ^{aA}
		10	-0.27 ± 0.009 ^{aA}	-0.30 ± 0.023 ^{aA}
		0	-0.20 ± 0.004 ^{aB}	-0.22 ± 0.020 ^{aB}
	hm3	20	0.27 ± 0.00 ^{aB}	0.29 ± 0.01 ^{bB}
		10	0.25 ± 0.00 ^{aA}	0.26 ± 0.01 ^{aA}
		0	0.31 ± 0.00 ^{aC}	0.32 ± 0.00 ^{bC}
Enthalpy (J/g)	$\Delta Hm1$	20	20.81 ± 1.73 ^{aA}	25.30 ± 2.18 ^{aA}
		10	38.32 ± 6.43 ^{aAB}	36.73 ± 1.51 ^{aAB}
		0	44.23 ± 3.11 ^{aB}	45.26 ± 4.84 ^{aB}
	$\Delta Hm2$	20	-23.96 ± 1.46 ^{aA}	-26.34 ± 0.32 ^{aA}
		10	-24.01 ± 1.21 ^{aA}	-24.15 ± 0.66 ^{aA}
		0	-16.13 ± 0.45 ^{aB}	-16.52 ± 1.56 ^{aB}
	$\Delta Hm3$	20	65.48 ± 0.11 ^{aA}	72.71 ± 0.59 ^{bA}
		10	76.02 ± 2.02 ^{aB}	80.23 ± 1.74 ^{aA}
		0	90.55 ± 0.33 ^{aC}	93.65 ± 3.21 ^{aB}
Crystallization	Months after exp. dates		SR	NSR
Peak temperature (°C)	20		-63.72 ± 0.06 ^{aA}	-62.15 ± 1.51 ^{aA}
	10		-63.57 ± 0.4 ^{aA}	-63.67 ± 0.26 ^{aA}
	0		-60.64 ± 0.01 ^{aB}	-60.71 ± 0.16 ^{aA}
Peak height (W/g)	20		-0.08 ± 0.003 ^{bC}	-0.10 ± 0.002 ^{aB}
	10		-0.09 ± 0.001 ^{bB}	-0.11 ± 0.003 ^{aB}
	0		-0.13 ± 0.001 ^{bA}	-0.14 ± 0.002 ^{aA}
Enthalpy (J/g)	20		-26.07 ± 1.79 ^{aA}	-28.29 ± 2.46 ^{aA}
	10		-28.6 ± 1.32 ^{aA}	-28.91 ± 0.56 ^{aA}
	0		-28.99 ± 1.34 ^{aA}	-31.21 ± 0.04 ^{aA}

SR: oil not clarified, with seed residue, NSR: oil clarified, with no seed residue. Values are given as mean $\pm$ SD ($n = 2$). Different letters (a, b) in the same row indicate a significant difference ($p \leq 0.05$). Different letters (A, B, C) in the same column within the same analysis and the same DSC parameters indicate a significant difference ($p \leq 0.05$).

Concerning the influence of seeds' age, the most distinctive differences were observed in the melting enthalpies (ΔHm) between oils from fresh (0 month) and older seeds (10 and 20 months) in both groups. The results showed that oils with better oxidative stability possessed higher enthalpies $\Delta Hm1$ and $\Delta Hm3$ than other oils from older seeds ($p \leq 0.05$). A continuous oxidation process was reported to affect the gradual decrement of unsaturated fatty acid in blended raspberry seed oil [24]. The decreased amount of unsaturated fatty

acid in oils from older seeds might be linked to the lower melting enthalpies, as presented in Table 3.

4. Conclusions

The removal of seed residue significantly reduced the quality of RSO, as indicated by higher p-anisidine value, lower DPPH, and lower OIT 120 °C in the NSR group. No distinctive differences were observed in the DSC melting and crystallization profile of raspberry seed oil from SR and NSR groups. Additionally, seed freshness was reported to affect the quality characteristics, DSC oxidative stability, and melting properties of raspberry seed oil from the SR and NSR groups. To conclude, the intended use of the final RSO product should be considered before removing the seed residue. The clarification process might be beneficial to improve the visual appearance of RSO, but it lowers both the oxidative stability of the oil and its shelf life. This work provided new information about the processing parameters in raspberry seed oil production, which is of benefit to seed oil producers.

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Thermal and spectroscopic profiles variation of cold-pressed raspberry seed oil studied by DSC, UV/VIS, and FTIR techniques

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ABSTRACT

The characteristics of six cold-pressed raspberry seed oils, investigated by melting and crystallization phase transition analysis using differential scanning calorimetry (DSC), UV–VIS and Fourier transform infrared (FTIR) spectra, as well as the fatty acid composition, was the aim of this study. Two groups of oils samples were investigated: commercial (I-group) and laboratory-pressed (L-group). UV/VIS and FTIR spectra of raspberry seed oil revealed important wavelength and wavenumber ranges to indicate qualitative differences between I- and L-group. Regardless of the scanning rates, DSC melting curves were more suitable for detecting differences in raspberry seed oils than crystallization profiles. Based on the Principal component analysis of UV–VIS, FTIR and DSC data two groups of oils were distinguished. Fatty acids by GC analysis confirmed differences between oils, since the commercial oils exhibited a lower ratio of *n*-3/*n*-6 fatty acids than L-group oils. The obtained results provide practical information on the usefulness of DSC profiles and FTIR spectra for the comprehensive characterization of cold-pressed raspberry seed oils, especially in the field of B2B industries that need rapid and non-targeted instruments to confirm the authenticity of the purchased product.

1. Introduction

The raspberry (*Rubus idaeus* L.) production in European Union (EU) was reported to increase up to 45 % in gross production value during the last 10 years (FAOSTAT, 2023). Raspberries can be consumed freshly or in the form of processed products, such as juice, jams, dried, or frozen raspberries. Raspberry processing generates large quantities of byproducts, mostly in the form of pomace and seeds. Due to its chemical composition and nutritive value, several studies reported the potency of valorized raspberry byproducts as nutritional supplements (Bredsdorff et al., 2015), cosmetics (Papaioanou et al., 2018), and food additives (Mildner-Szkudlarz et al., 2016). Apart from the nutritional value,

raspberry byproduct, particularly the seed contains a high percentage of oil. Raspberry seed oil yield was reported to reach 23 %, depending on the parameters and method of extraction used (Teng et al., 2016). As a niche product, cold-pressed raspberry seed oil was sold at higher prices on the market (Przykaza et al., 2021). In the second quarter of 2023, the price of raspberry seed oil ranged from 20 to 25 EUR per 100 mL. Although it is a relatively new product, interest in its production is continuously growing due to the lower cost of raw materials and high profitability.

As with other highly valued edible oils, raspberry seed oil is prone to adulteration. Highly valued edible oils are frequently adulterated with refined oil, less expensive vegetable oil (e.g. soybean and palm oil), and

Abbreviations: SFA, saturated fatty acid; MUFA, mono-unsaturated fatty acid; PUFA, polyunsaturated fatty acid; UFA, unsaturated fatty acid; AI, atherogenicity index; TI, thrombogenicity index; HH, hypo- and hypercholesterolemic ratio.

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even with the addition of chemicals to mask fraud (Karbasian et al., 2015; Roca et al., 2010). In addition to the fraud, a study investigated the authenticity of avocado oil sold on the US market and reported that the adulteration level with soybean oil nearly reach 100 % in a product labelled “extra-virgin” and “refined” (Green and Wang, 2020). In the newly marketed oil industry, the consumer remains unprotected from possible fraud because there is no enforceable regulation to control the quality and the authenticity of oil, as Codex Alimentarius regulations currently only control the properties of some widely consumed oils (FAO, 1999). In response to the emerging problems, an urgent development in the quality standard for raspberry seed oil is needed in order to protect the consumer, honest producers in the B2B chain of edible oil industry as a whole.

DSC and FTIR were reported to exhibit strong capabilities in the field of authenticity assessment in recent years (Cebi et al., 2021; Tomaszewska-Gras, 2016a). DSC is able to map the fingerprint spectrum based on the oil and fat's thermal behaviour. Not limited to fingerprints, FTIR is utilized to investigate the quality deterioration of oil by tracking the absorption changes. Both instruments are fast, chemical-free, non-destructive, and reliable, which makes them suitable for rapid authenticity monitoring. To the best of our knowledge, no study has comprehensively reported the DSC phase transition and FTIR spectroscopy profile of cold-pressed raspberry seed oil.

Studies focusing on the method of extraction and general characteristics of raspberry seed oil were previously reported (Oomah et al., 2000; J. Parry and Yu, 2004; Pavlić et al., 2020). The oxidative stability of raspberry seed oil by DSC was also reported in some studies (Micić et al., 2014; Rajagukguk et al., 2023a). However, there is still a need to gain an understanding of the natural characteristics and quality range of raspberry seed oil sold on the market and how it differs from freshly prepared samples. Additionally, the DSC phase transition of hexane-extracted raspberry seed oil was briefly reported in another study (Piasecka et al., 2021). Most of the raspberry seed oils sold on the market were cold-pressed oil, the absence of chemical involvement makes cold-pressed oil safe for direct use or consumption. Hence, a comprehensive DSC study on cold-pressed raspberry seed oil is still needed to fill the knowledge gap of the marketed products.

This study covers the aspects of phase transition and spectroscopy study of raspberry seed oil by DSC, FTIR and UV–VIS techniques. The study aimed to investigate the natural characteristics in fatty acid composition by GC, melting-crystallization behaviour, and also spectroscopy profile by discriminating marketed cold-pressed raspberry oil with a freshly prepared sample. Secondly, the study also aimed to explore the most suitable parameters to assess raspberry seed oil authenticity by DSC. Studies were conducted by observing the thermal behaviour of the oils at various scanning rates. The study is the first attempt to utilise the whole FTIR spectra and DSC melting-crystallization curves for the quality and authenticity determination of raspberry seed oils.

2. Material and methods

2.1. Chemicals and reagents

1,1-diphenyl-2-picrylhydrazyl (DPPH), Trolox, sodium methoxide were supplied from Sigma-Aldrich (Steinheim, Germany). Isooctane (pure p.a.), n-hexane (95 % for HPLC) and ethyl acetate (99.8 %) were obtained from POCH, Avantor Performance Materials Poland S.A. (Gliwice, Poland). The Supelco 37 component fatty acid methyl ester (FAME) reference material was purchased from Sigma-Aldrich (Steinheim, Germany).

2.2. Raspberry seed oil

In this study, six cold-pressed raspberry seed oils were used: commercially purchased (I1, I2, I3) and laboratory-pressed oils (L1, L2,

L3). Samples of industrial oils were purchased from the market in Poland in the form of ready-to-use oil. For the laboratory-pressed oils three different batches of raspberry seeds were obtained from the company GreenField Sp. z o.o. (Poland). To protect the seeds from oxidation, they were stored in an aluminium bag and kept in the dark at 4 °C prior to cold-pressing. Raspberry seeds were pressed using a screw-pressing machine (Counter Intelligence Oilpresso, Netherlands) at 45 °C and collected directly after pressing. All of the samples were kept in brown-glass bottles and purged with nitrogen gas (99.99 % purity). These samples were stored in the freezer at –80 °C until analysis. The acid value of samples were 3.71 ± 0.03 , 3.71 ± 0.06 , 13.56 ± 0.25 for I-group (I1, I2, I3) and 6.59 ± 0.03 , 10.06 ± 0.31 , 5.17 ± 0.03 mg KOH/g oil for L-group (L1, L2, L3), respectively.

2.3. Fatty acid composition

Fatty acid composition was determined by gas chromatography (GC), following the method described by AOCS (AOCS, 2005). The sample preparation started by adding 1 mL of hexane (>99 %, Avantor, Gliwice, Poland) to the cold-pressed oil ($\pm 50 \mu\text{L}$), followed by 1 mL addition of 0.4 M sodium methoxide (Merck, Darmstadt, Germany). The samples were then thoroughly mixed and left to stand for 15 min. The reaction was stopped by the addition of 5 mL distilled water. A hexane-containing upper layer was observed in the sample mixture, and the upper layer (± 0.5 mL) was then transferred into GC-vial and proceed for analysis. The gas chromatography analyses were carried out using Trace 1300 with a FID detector (Thermo Scientific, Waltham, MA, USA) equipped with a SP TM-2650 capillary column (100 m $\times$ 0.25 mm $\times$ 0.2 μm) (Supelco, Bellefonte, PA, USA). The analyses were performed in splitless mode using hydrogen gas (1.5 mL/min) as the carrier. The initial oven temperature was 160 °C and was maintained for 1 min, then heated by 6 °C/min until the temperature reached 220 °C. The temperature of the inlet and detector were 240 °C. Retention time was determined based on the comparison between the samples' retention time with reference material of the 37 Component FAME Mix (Supelco).

Some indices to refer the health implication of fatty acid, such as atherogenicity, thrombogenicity, hypo- and hypercholesterolemic, were calculated (Chen and Liu, 2020). The atherogenicity index (AI) indicates the relationship between the total of saturated and unsaturated fatty acid, it is calculated as follows:

$$\text{AI} = [\text{C12:0} + (4 \times \text{C14:0}) + \text{C16:0}] / \sum \text{UFA} \quad (1)$$

The thrombogenicity index (TI) indicates the tendency of fatty acid to for clots in blood vessels, it is calculated as follows:

$$\text{IT} = (\text{C14:0} + \text{C16:0} + \text{C18:0}) / [(0.5 \times \sum \text{MUFA}) + (0.5 \times \sum n-6 \text{ PUFA}) + (3 \times \sum n-3 \text{ PUFA}) + (n-3/n-6)] \quad (2)$$

where:

The hypo- and hypercholesterolemic index (HH) indicates the relationship between hypocholesterolemic and hypercholesterolemic fatty acid, it is calculated as follows:

$$\text{HH} = (\text{cis-C18:1} + \sum \text{PUFA}) / (\text{C12:0} + \text{C14:0} + \text{C16:0}) \quad (3)$$

2.4. Melting and crystallization profile by DSC

Melting and crystallization profiles of raspberry seed oil were determined following the method described by Tomaszewska-Gras (2016b) with a minor modification. The melting and crystallization analyses were carried out using a PerkinElmer differential scanning calorimeter DSC 8500 (Waltham, Massachusetts, USA) with an Intracooler II and Pyris software. The instrument was calibrated using indium (m.p. 156.6 °C, $\Delta H_f = 28.45 \text{ J/g}$) and n-dodecane (m.p. –9.65 °C, $\Delta H_f = 216.73 \text{ J/g}$). Raspberry seed oil samples (6–7 mg) were weighed into

aluminium pans (PerkinElmer, No. 0219–0062, Waltham, Massachusetts, USA) and hermetically sealed. An empty and sealed aluminium pan was used as the reference. The samples were heated at 30 °C for 5 min before the analysis took place, then cooled with scanning rate 1 and 2 °C/min from temperature 30 to –67 °C, while the melting curve was taken from –67–30 °C at a heating rate of 1, 2, 5 °C/min. Peak temperature (T), peak height (h) and enthalpy (ΔH) of melting and crystallization were measured from the cooling or heating curves. All measurements were conducted in duplicate.

2.5. UV/Vis spectra

The absorptivity of the oil was measured on a Cary 1E spectrophotometer (Varian, Belrose, Australia) based on the AOCS method Ch-591 (AOCS, 2009). The sample was filtered through filter paper at ambient temperature to avoid impurities. The sample without impurities was then thoroughly mixed with pure isooctane (2,2,4-trimethylpentane) at a 1:1 ratio. The oil absorptivity was measured at a wavelength range from 200 to 750 nm using a quartz cuvette. The measurement was performed in triplicate.

2.6. FTIR spectroscopy

The FTIR spectra of raspberry seed oils were obtained using a Spectrum Two FT-IR spectrometer equipped with a Universal ATR with a diamond crystal (PerkinElmer, Waltham, MA, USA). The data were collected over a spectral range of 4000–500 cm^{-1} (32 scans/sample or background) at 4 cm^{-1} resolution in reflectance mode. The spectrum of the crystal surface was recorded and used as a background, the analysis was carried out at room temperature. Raspberry seed oil ($\pm 50 \mu\text{L}$) was uniformly dropped onto the surface of ATR crystal for the spectral data acquisition. The spectra were recorded two times for each sample. The outer surface of ATR crystal was cleaned with ethanol after each acquisition.

2.7. Statistical analysis

All statistical tests with the level of significance set at 5 % were performed using Statistica 13.3 software (StatSoft, Tulsa, Oklahoma, USA). ANOVA with repeated measures was applied to estimate the effect of the variable on the parameters. The homogenous groups were shown using HSD Tukey's test. Additionally, principal component analysis (PCA) and cluster analysis (CA) were performed as exploratory methods to show the relationships between variables/objects and detect some patterns between variables and objects. The analysis also enables the dataset to be reduced from a higher to a lower dimensional level.

3. Results and discussion

3.1. Fatty acid composition

Fatty acid composition of cold-pressed raspberry seed oils is presented in Table 1. Raspberry seed oil is mainly composed of palmitic (C16:0), oleic (C18:1), linoleic (C18:2), as well as α -linolenic (C18:3n-3) acids. Linoleic acid contributes to the largest proportion of fatty acid in raspberry seed oil, then followed by α -linolenic acid. The results showed that the L-group (L1, L2, L3) and sample I3 contained a similar percentage of dominant fatty acids i.e. C16:0 in the range of 1.83–2.33 %, C18:1 in the range 11.29–11.92 %, C18:2 in the range 52.14–56.2 % and C18:3n-3 in the range of 28.23–32.36 %, while for the rest of samples (I1 and I2) the ranges were: 4.01–4.82; 16.46–19.57; 61.49–61.92 %; 10.06–14.94 %, respectively. The fatty acid composition of oil samples L1, L2, L3 and I3 was in accordance with the results reported in other studies (Fotschki et al., 2015; Micić et al., 2015a; John Parry et al., 2005; Pieszka et al., 2015). However, we did not find any reports with such a low content of α -linolenic (C18:3n-3) as was determined in the samples

Table 1

Fatty acid composition expressed as total fatty acid (%) of industrial raspberry seed oil (I1, I2, I3) and laboratory-pressed raspberry seed oil (L1, L2, L3).

Fatty acid ^a	Raspberry seed oils (%)					
	I1	I2	I3	L1	L2	L3
14:0	0.04 $\pm$ 0.00 ^c	0.05 $\pm$ 0.00 ^c	0.00 $\pm$ 0.00 ^a	0.02 $\pm$ 0.01 ^{ab}	0.02 $\pm$ 0.01 ^{ab}	0.02 $\pm$ 0.00 ^b
16:0	4.01 $\pm$ 0.01 ^d	4.82 $\pm$ 0.00 ^e	1.83 $\pm$ 0.11 ^a	1.94 $\pm$ 0.00 ^{ab}	2.08 $\pm$ 0.04 ^b	2.33 $\pm$ 0.01 ^c
16:1	0.06 $\pm$ 0.00 ^a	0.07 $\pm$ 0.00 ^a	0.07 $\pm$ 0.01 ^a	0.05 $\pm$ 0.00 ^a	0.06 $\pm$ 0.00 ^a	0.07 $\pm$ 0.01 ^a
18:0	1.82 $\pm$ 0.00 ^d	2.29 $\pm$ 0.01 ^e	0.66 $\pm$ 0.00 ^a	0.66 $\pm$ 0.01 ^b	0.70 $\pm$ 0.01 ^b	0.90 $\pm$ 0.01 ^c
18:1	16.46 $\pm$ 0.02 ^c	19.57 $\pm$ 0.03 ^d	11.29 $\pm$ 0.01 ^a	11.71 $\pm$ 0.00 ^b	11.79 $\pm$ 0.13 ^b	11.92 $\pm$ 0.03 ^b
18:2 n-6	61.49 $\pm$ 0.03 ^e	61.92 $\pm$ 0.01 ^f	55.09 $\pm$ 0.11 ^c	56.2 $\pm$ 0.04 ^d	52.14 $\pm$ 0.14 ^a	54.77 $\pm$ 0.00 ^b
18:3 n-3	14.94 $\pm$ 0.04 ^b	10.06 $\pm$ 0.01 ^a	30.15 $\pm$ 0.04 ^e	28.23 $\pm$ 0.01 ^c	32.36 $\pm$ 0.16 ^f	29.02 $\pm$ 0.02 ^d
18:3 n-6	0.03 $\pm$ 0.00 ^a	0.03 $\pm$ 0.01 ^a	0.08 $\pm$ 0.00 ^b	0.08 $\pm$ 0.01 ^b	0.08 $\pm$ 0.00 ^b	0.12 $\pm$ 0.00 ^c
20:0	0.22 $\pm$ 0.00 ^a	0.22 $\pm$ 0.00 ^a	0.34 $\pm$ 0.02 ^b	0.30 $\pm$ 0.02 ^b	0.38 $\pm$ 0.01 ^c	0.50 $\pm$ 0.01 ^c
20:1	0.13 $\pm$ 0.03 ^{ab}	0.14 $\pm$ 0.01 ^{ab}	0.15 $\pm$ 0.01 ^{ab}	0.14 $\pm$ 0.01 ^{ab}	0.10 $\pm$ 0.01 ^{ab}	0.09 $\pm$ 0.00 ^a
20:2	0.02 $\pm$ 0.00 ^b	0.02 $\pm$ 0.00 ^b	0.00 $\pm$ 0.00 ^a	0.05 $\pm$ 0.01 ^c	0.04 $\pm$ 0.00 ^c	0.04 $\pm$ 0.00 ^c
22:0	0.39 $\pm$ 0.01 ^b	0.47 $\pm$ 0.00 ^c	0.12 $\pm$ 0.03 ^a	0.10 $\pm$ 0.00 ^a	0.11 $\pm$ 0.00 ^a	0.08 $\pm$ 0.00 ^a
24:0	0.12 $\pm$ 0.01 ^b	0.14 $\pm$ 0.01 ^b	0.05 $\pm$ 0.01 ^a	0.04 $\pm$ 0.01 ^a	0.05 $\pm$ 0.01 ^a	0.04 $\pm$ 0.00 ^a
22:5	0.06 $\pm$ 0.00 ^b	0.05 $\pm$ 0.00 ^b	0.10 $\pm$ 0.01 ^c	0.01 $\pm$ 0.00 ^a	0.01 $\pm$ 0.00 ^a	0.01 $\pm$ 0.00 ^a
22:6	0.23 $\pm$ 0.01 ^d	0.18 $\pm$ 0.00 ^c	0.09 $\pm$ 0.01 ^a	0.51 $\pm$ 0.01 ^e	0.12 $\pm$ 0.01 ^{ab}	0.13 $\pm$ 0.01 ^b
ΣSFA	6.59	7.99	2.99	3.04	3.33	3.86
ΣMUFA	16.65	19.78	11.51	11.90	11.95	12.08
ΣPUFA	76.76	72.25	85.50	85.06	84.74	84.08
n-3/n-6	0.24	0.16	0.55	0.50	0.62	0.53
UFA/ SFA	14.18	11.52	32.44	31.89	29.08	24.91
AI	0.04	0.05	0.02	0.02	0.02	0.03
TI	0.07	0.10	0.02	0.02	0.02	0.03
HH	29.97	18.80	52.79	49.21	46.00	40.86

Values are given as mean $\pm$ SD (n = 2). Different letters (a-f) in the same row indicate significant difference ($p \leq 0.05$). I: industrial raspberry seed oil (I1, I2, I3), L: laboratory-pressed raspberry seed oil (L1, L2, L3).

^a SFA: saturated fatty acid, MUFA: mono-unsaturated fatty acid, PUFA: poly-unsaturated fatty acid, UFA: unsaturated fatty acid, AI: atherogenicity index, TI: thrombogenicity index, HH: hypo- and hypercholesterolemic ratio.

I1 and I2. These oils (I1, I2) also exhibited a lower ratio of n-3/n-6 compared to the rest of the samples, which was caused mainly by the percentage of α -linolenic acid, which was more than 50 % lower in comparison with other samples (I3, L1, L2, L3). Additionally, sample I1 and I2 were reported to have higher amounts of saturated (C16:0, C18:0) and monounsaturated fatty acids (C18:1). Although the variation in fatty acid composition might have been caused by the difference in soil condition, cultivation region and the harvest season (Yilmaz and Güneşer, 2017), there are no papers reporting such a high content of these fatty acids.

The abundant amount of polyunsaturated fatty acid in raspberry seed oil indicates its functionality as a potential health-promoting agent, such as cardioprotective food supplements (Jurgoński et al., 2018), natural cosmetics, and pharmaceutical use (Ispiryan et al., 2021). The range of AI (0.02–0.05) and TI (0.02–0.1) in raspberry seed oils were relatively lower than for instance for camelina seed oil, which is also rich in unsaturated fatty acids (Ratusz et al., 2018). Additionally, the HH ratio of raspberry seed oils (40.86–52.79) is higher than most of reported food materials (except the sample I1 and I2) (Chen and Liu, 2020). In short, low AI, IT indices and high HH ratio indicate the high nutritional quality of raspberry seed oil.

3.2. UV/Vis spectra of cold-pressed raspberry seed oils

Ultraviolet and visible spectra of cold-pressed raspberry seed oil at wavelengths from 200 nm to 750 nm are shown in Fig. 1. They indicate the presence of aromatic and other organic compounds, which have been detected in other cold-pressed oils (Popa et al., 2020). The maximum at 216 nm is related to the presence of $\pi-\pi^*$ transitions. A range between 220 and 250 nm indicates the presence of carboxylic and phenolic acid moieties, between 260 and 320 nm the presence of an aromatic ring structure of compounds and the presence of phenolic acids such as gallic, caffeic, and ferrulic. In the range from 400 nm and 500 nm, characteristic spectra were observed with three maxima at 420 nm, 443 nm and 473 nm, ascribed to carotenoids, responsible for yellow colour of oil. Finally, at the long wave range of the spectrum, the spectra with maxima at 670 nm indicates the presence of chlorophyll pigments, although the peaks from samples were barely seen, which can indicate that they will not act as prooxidants in the presence of light. Generally, the measured UV/Vis spectra of cold-pressed oil from raspberry seeds were similar to that presented by Oomah et al. (2000). However, the spectra presented in this study, exhibit differences between samples in the aforementioned regions, first of all, indicating differences in the

concentrations of compounds. While the band at 234 nm indicates the presence of carboxylic and phenolic acid moieties, we have to be aware that it also serves as an indicator of conjugated dienes as markers of oxidation. The elevated absorbance at 234 nm for samples I2, L1 and L2 may indicate the presence of oxidized fatty acids in those samples, especially considering that we observe also a peak at 270 nm, characteristic for triene formation. The observed maxima in the spectral range between 260 nm and 340 nm indicate the presence of tocopherols and phenolic acids. HPLC studies have confirmed that γ -tocopherol is the main isomer, present in cold-pressed raspberry oil (Rajagukguk et al., 2023b). The fraction of carotenoids and chlorophyll pigments in edible oil were detected at 470 nm and 670 nm, as depicted in Fig. 1C, which is in line with other studies (Borello and Domenici, 2019). As mentioned above, between 400 and 500 nm the presence of carotenoids is clearly seen (Fig. 1C). However, samples I2 and L2 are lacking in carotenoids, which were present in the other samples. The spectra around 670 nm indicate the presence of chlorophyll pigment, but the amount is so negligibly low that even the colour of the oil sample does not show any greenish hue. The results showed that raspberry seed oils from both groups have a negligible absorption level that represented carotenoid (<0.04) and chlorophyll pigments (<0.02). However, due to high

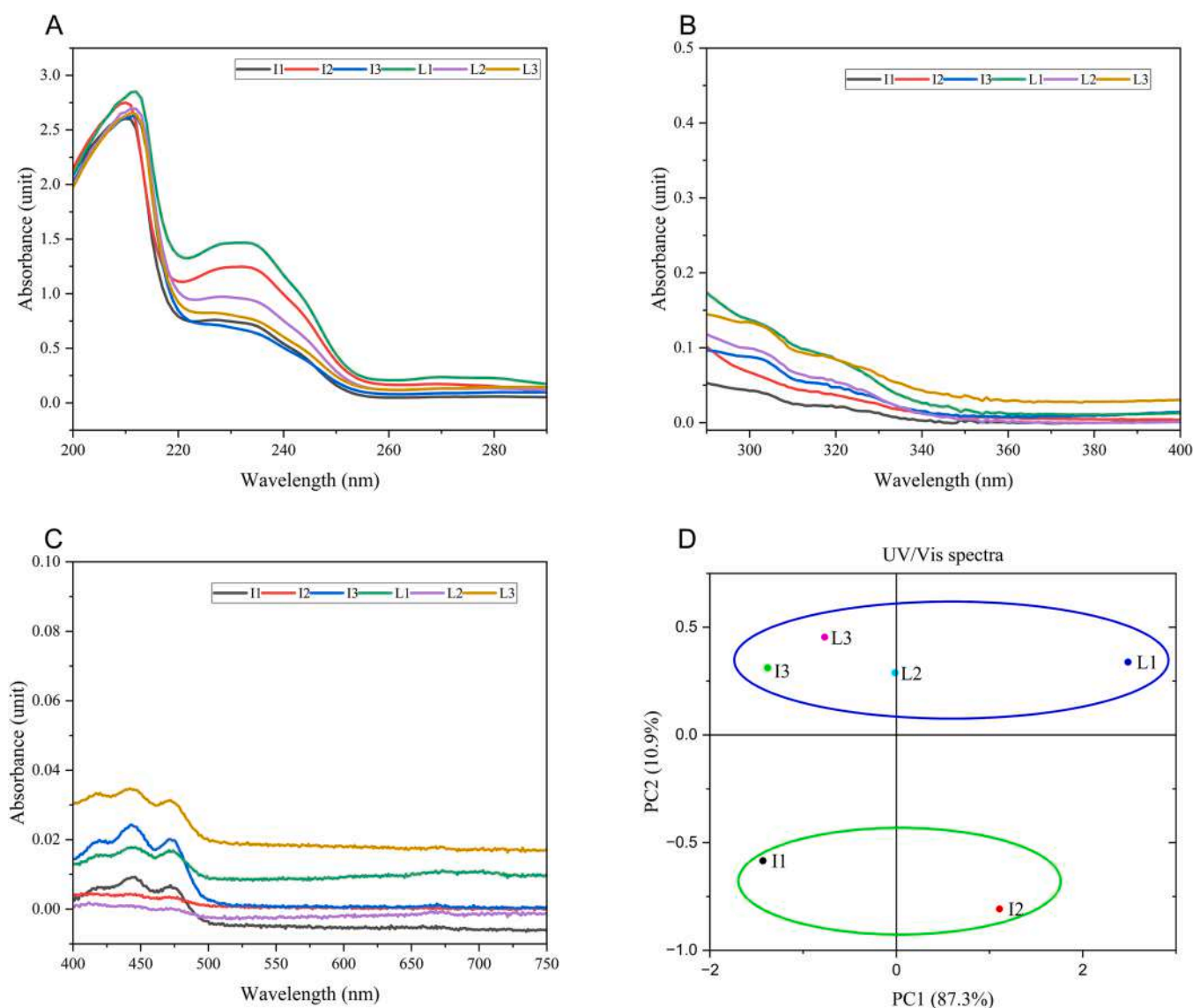


Fig. 1. Ultra violet/visible spectra of raspberry seeds oil diluted in 1:1 solution, (A) at wavelength 200 – 290 nm, (B) 290 – 400 nm, and (C) 400 – 750 nm, (D) scatter plots for PC1 and PC2 analyses of UV/Vis spectra. I: industrial raspberry seed oil (I1, I2, I3), L: laboratory-pressed raspberry seed oil (L1, L2, L3).

extinction coefficient of carotenoids its presence gives a yellowish colour to the oil samples.

Based on similar characteristics in the absorptivity of all the oils investigated, the whole UV–VIS spectra of cold-pressed raspberry seed oils were subjected to Principle Component Analysis (PCA), as depicted in Fig. 1D. All oils samples were divided into two groups, i.e. the first group consisted of laboratory-pressed oil (L1, L2, L3) with one commercial (I3), while rest commercial samples (I1, I2) belonged to another group.

3.3. FTIR spectra of cold-pressed raspberry seed oils

The FTIR spectra of raspberry seed oil were measured at the range of 4000–500 cm^{-1} , as shown in Fig. 2A. The positions of the peak maxima reflect the functional groups of all compounds in oils (Embaby et al., 2022). Their intensities, widths and ratios of intensities between specific peaks provide information regarding the structure and composition of the oil. The measured FTIR spectrum indicates the presence of groups characteristic for fatty acids and triacylglycerols, i.e. the groups $-\text{CH}_3$, $-\text{CH}_2$, $-\text{C}=\text{O}$, $-\text{OH}$, and the *cis* and *trans* structures of the carbon double bonds ($\text{C}=\text{C}$). The analysis of the position of characteristic spectral bands makes it possible to evaluate the composition of the oil that is obtained, especially in terms of its oxidation and the ratio of saturated to unsaturated fatty acids. A good measure of the presence of unsaturated fatty acids was the calculation of the peak ratio of 3011/2925 cm^{-1} , which expresses the presence of C-H bonds involved in the *cis* $\text{C}=\text{C}$ bond. These values are 0.23–0.24 for L-group oil samples, 0.24 for the I3 and 0.19 and 0.17 for samples I1 and I2, respectively. There is a significant correlation between the UFA/SFA ratio calculated from fatty acid composition (Table 1) and ratio of 3011/2925 cm^{-1} , as shown in Fig. 2. This indicator was used for testing adulterations of olive oil with soybean, sunflower or rapeseed oil (Poiana et al., 2015). The band at 1712 cm^{-1} (Fig. 2C), is related to another important parameter, which qualitatively expresses the presence of free fatty acids in the sample (Dong et al., 2015). The highest intensity of this peak was observed for sample I3 and lowest for I1. This finding is in line with the acid value measurement (Section 2.2), for which the highest value was for I3 sample (13.56 mg KOH/g oil) and the lowest for I1 (3.71 mg KOH/g oil). Additionally, its position indicates the presence of acyl groups of linoleic and α -linolenic acids in oil, since the presence of oleic acid would shift the maximum towards lower energy. Another important bands, used in analysis of oils quality are located at 966 cm^{-1} , linked to the presence of *trans* double bonds, and at 721 cm^{-1} , related to *cis* double bonds. If the sample is exposed to excessive heat or oxidation, *cis-trans* isomerization can occur, which is manifested by the disappearance of double bonds in *cis* conformation (Borchman and Sinha, 2002; Cebi et al., 2017; Valdés et al., 2015). In our study small differences at the 966 cm^{-1} band between samples' spectra have been observed (Fig. 2B). It can be noticed that I-group spectra exhibit a peak at 987 cm^{-1} associated with bending vibration of C-H *trans* and *cis* conjugated diene groups of hydroperoxides, which can be connected with first step of oxidation of those oils. This band is missing in the spectra of the L group, but there is the band at 966 cm^{-1} , indicating the presence of *trans* unsaturated fatty acids. However, if we compare the band at 721 cm^{-1} , related to *cis* double bonds of unsaturated fatty acids, there is not a change within a group. Thus, it may be concluded that the spectra do not indicate a significant presence of oxidized fatty acids in the oils. The small differences observed in oil samples might have been caused by the initial conditions of the raspberry seeds. It should be taken into account that raspberry seeds are in fact a by-product and the process of preparing seeds for pressing takes time. Therefore the seeds taken for pressing oil are never completely fresh. Thus, the lower content of *trans*-conjugated dienic compound at 988–950 cm^{-1} and lower free fatty acids content at 1712 cm^{-1} for samples I1–I2 might also indicate the partial refining process of oils. Additionally, for L-group oils higher absorbance was observed around 3470 cm^{-1} (Fig. 2D), which can correspond to the

presence of the hydroxyl group ($-\text{OH}$) due to the moisture in the oil (Embaby et al., 2022).

Based on the FTIR spectra, the PCA method was able to differentiate the samples into 3 clusters (Fig. 2E). The first cluster consisted of L-group samples, followed by two I-group samples (I1, I2), and last with sample I3 as the outlier. To the best of our knowledge, no study has ever reported the general FTIR profile of raspberry seed oil and the feasibility of FTIR to characterise raspberry seed oil. The reported FTIR spectral data of cold-pressed raspberry seed oil will be valuable for the rapid quality assessment and characterization of oils.

3.4. DSC melting and crystallization profiles

The thermal properties of raspberry seed oil were determined based on the melting and crystallization profiles presented in Fig. 3. Tables 2 and 3 present the DSC parameters determined from the crystallization and melting curves, which include: peak temperatures (T_c , T_{m1} , T_{m2} , T_{m3}), peak height (h_c , h_{m1} , h_{m2} , h_{m3}), and enthalpy of phase transition (ΔH_c , ΔH_{m1} , ΔH_{m2} , ΔH_{m3}). The thermal properties of raspberry seed oils were measured at scanning rates 1, 2, 5 $^{\circ}\text{C}/\text{min}$ (Fig. 3). Generally, the thermal profile of raspberry seed oils from L-group and sample I3 are similar with melting and crystallization profiles of the same oil reported in other studies (Micić et al., 2015b; Oomah et al., 2000). The similarities were mainly found in the peak temperatures of melting ($T_{m1} = \pm -45^{\circ}\text{C}$, $T_{m2} = \pm -43^{\circ}\text{C}$, $T_{m3} = \pm -23^{\circ}\text{C}$) and crystallization ($T_c = \pm -62^{\circ}\text{C}$) curves at 1 $^{\circ}\text{C}/\text{min}$ (Oomah et al., 2000). Additionally, both melting and crystallization curves from samples L1, L2, L3, and I3 are visually similar with the curves at 2 $^{\circ}\text{C}/\text{min}$ reported in another study (Micić et al., 2015b).

The cooling curves of all samples are presented in Fig. 3A (scanning rate 1 $^{\circ}\text{C}/\text{min}$) and Fig. 3C (scanning rate 2 $^{\circ}\text{C}/\text{min}$). In cooling curves, one exothermic peak was detected at 1 $^{\circ}\text{C}/\text{min}$ at temperature around -62°C . The differences in the shape of peaks can be seen between scanning rate 1 and 2 $^{\circ}\text{C}/\text{min}$, since for scanning rate 1 $^{\circ}\text{C}/\text{min}$ all phase transitions reached the end temperature, in contrary to the curves for scanning rate 2 $^{\circ}\text{C}/\text{min}$, for which only for one sample (I1) whole phase transition can be seen. For the rest of samples, lower final temperatures should be set, but because of equipment limitation, this was not possible. The lowest possible temperature was -65°C . In Table 2, the results of peak temperature and peak height and enthalpy are shown. Significant differences in peak temperature (T_c) among all samples were shown in cooling curves at 1 $^{\circ}\text{C}/\text{min}$ ($p \leq 0.05$). Higher T_c was dominated with the I-group (I1, I2), followed by L3, and the rest of the samples. Peak temperatures are affected by the degree of unsaturation of fatty acids and the length of the fatty acid chain (Marikkar et al., 2013). Sample I1 and I2 showed the highest T_c among all samples, and this corresponds to their higher percentage of SFA and MUFA compared to sample I3 and L-group, which contained a higher percentage of PUFA, especially with three double bonds (Table 1). Higher cooling rates resulted in a lower T_c . These results are in agreement with the study of cooling rate variations effect on the DSC crystallization profile of flaxseed oil (Tomaszewska-Gras et al., 2021).

On the melting curves, presented in Fig. 3B (scanning rate 1 $^{\circ}\text{C}/\text{min}$), Fig. 3D (scanning rate 2 $^{\circ}\text{C}/\text{min}$) and Fig. 3E (scanning rate 5 $^{\circ}\text{C}/\text{min}$), three peaks were detected, two endothermic (T_{m1} , T_{m3}) and one exothermic (T_{m2}). The results showed (Table 3), that higher heating rates result in the higher peak temperatures (T_m) as well as higher peaks (h_m). For scanning rate 1 $^{\circ}\text{C}/\text{min}$ first and third endothermic peak appeared at $T_{m1} = \pm -40^{\circ}\text{C}$, $T_{m3} = \pm -21^{\circ}\text{C}$ and one exothermic peak at $T_{m2} = \pm -37^{\circ}\text{C}$, for scanning rate 2 $^{\circ}\text{C}/\text{min}$, three peaks were located at temperatures $T_{m1} = \pm -38^{\circ}\text{C}$, $T_{m2} = \pm -35^{\circ}\text{C}$, $T_{m3} = \pm -20^{\circ}\text{C}$, while for scanning rate 5 $^{\circ}\text{C}/\text{min}$ they were detected at higher temperatures i.e. $T_{m1} = \pm -37^{\circ}\text{C}$, $T_{m2} = \pm -29^{\circ}\text{C}$, $T_{m3} = \pm -17^{\circ}\text{C}$, except for two samples (I1, I2), for which all three peaks were endothermic and no exothermic peak was observed. Generally, when comparing two groups of oils i.e. I and L (Table 3), I-group

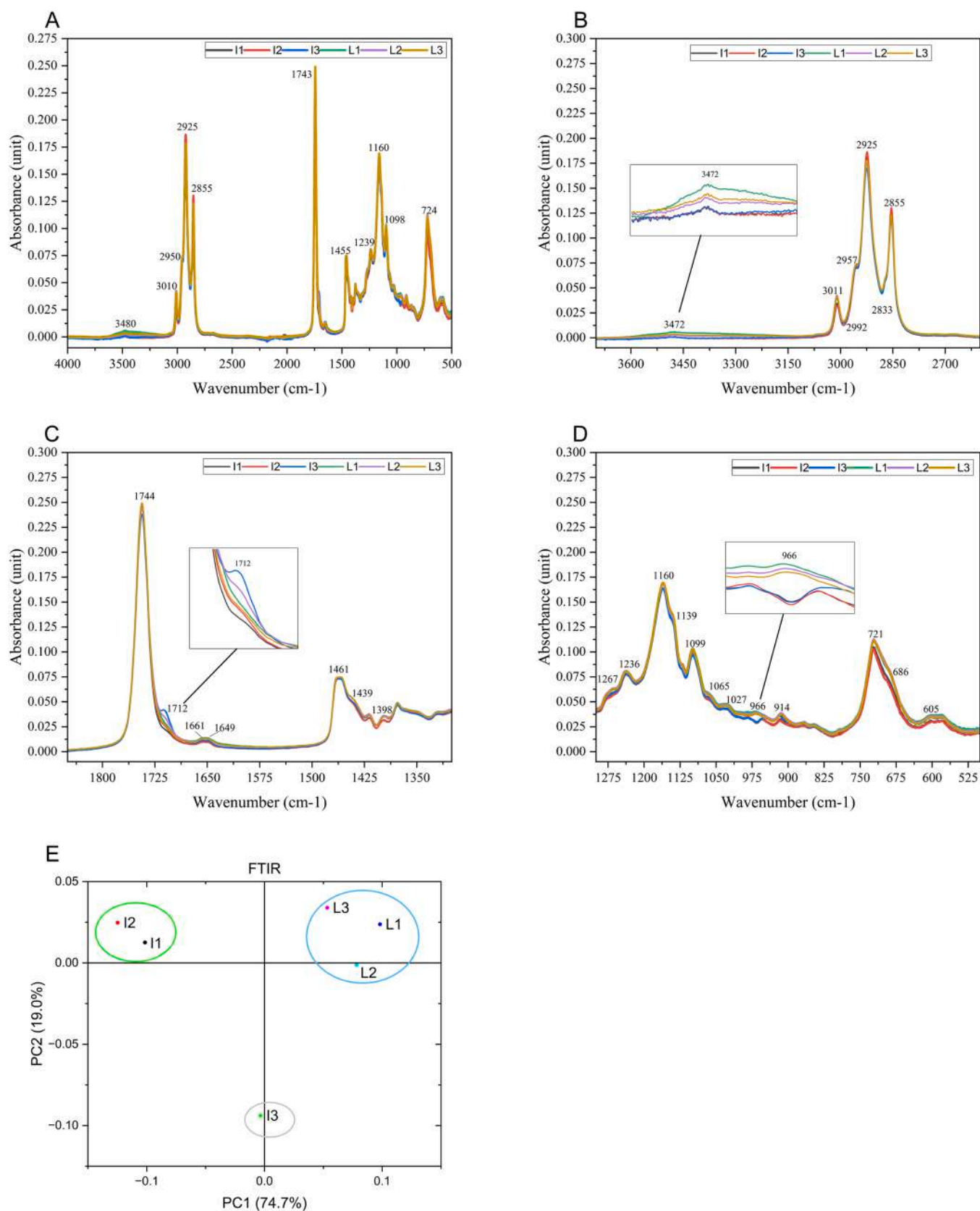


Fig. 2. FTIR spectra of raspberry seed oils, (A) ranging from 4000 to 500 cm^{-1} , (B) ranging from 3600 to 2700 cm^{-1} , (C) ranging from 1900 to 1370 cm^{-1} , (D) ranging from 1275 to 525 cm^{-1} , (E) scatter plots for PC1 and PC2 analyses of FTIR spectroscopy. I: industrial raspberry seed oil (I1, I2, I3), L: laboratory-preserved raspberry seed oil (L1, L2, L3).

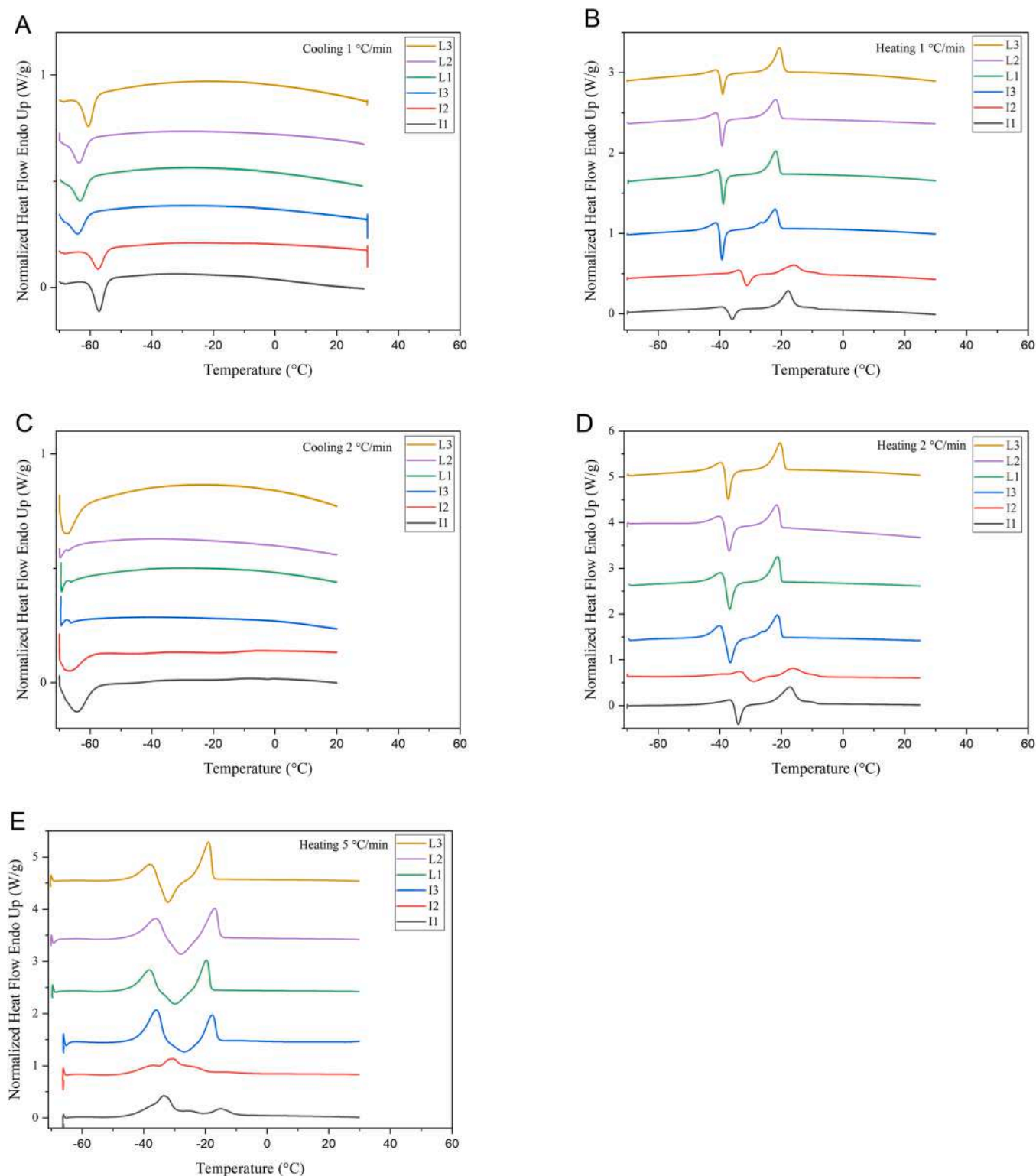


Fig. 3. DSC phase transition curves of raspberry seed oil (A) crystallization curves at 1 °C/min, (B) melting curves at 1 °C/min, (C) crystallization curves at 2 °C/min, (D) melting curves at 2 °C/min, (E) melting curves at 5 °C/min. I: industrial raspberry seed oil (I1, I2, I3), L: laboratory-pressed raspberry seed oil (L1, L2, L3).

samples, except I3, exhibited the highest T_m at heating rates 1 and 2 °C/min ($p \leq 0.05$), while sample I3 mostly exhibited a similar thermal profile with L-group samples ($p > 0.05$). In raspberry seed oil, the best peak resolution was obtained at heating rates 1 °C/min (Fig. 3B). Unfortunately, as the heating rate increased, particular peaks from sample I1 and I2 became less defined (Fig. 3E). Based on these results, it can be assumed that curves obtained at heating rate 1 °C/min could show small

differences between oils and could be used as the fingerprints of the raspberry seed oil for authenticity assessment. However, it is important to establish a typical curve for genuine raspberry seed oil, since differences even between cultivars could be detected at scanning rate 1 °C/min, as was shown for flaxseed oil (Tomaszewska-Gras et al., 2021). It is important to note that lower heating rates do not necessarily give the best resolution for authenticity assessment in every oil. In the case of

Table 2

DSC parameters of crystallization curves for raspberry seed oil for cooling rate 1 °C/min.

	Peak temperature (°C) - T _c	Peak height (W/g) - h _c	Enthalpy (J/g) - ΔH _c
I1	-57.18 ± 0.08 ^c	-0.15 ± 0.01 ^a	-34.15 ± 0.63 ^b
I2	-57.39 ± 0.01 ^c	-0.11 ± 0.00 ^c	-27.98 ± 0.98 ^b
I3	-63.83 ± 0.08 ^a	-0.09 ± 0.00 ^{de}	-25.87 ± 0.46 ^b
L1	-63.72 ± 0.06 ^a	-0.08 ± 0.00 ^c	-26.07 ± 1.79 ^a
L2	-63.57 ± 0.40 ^a	-0.09 ± 0.00 ^d	-28.60 ± 1.32 ^b
L3	-60.64 ± 0.01 ^b	-0.13 ± 0.00 ^b	-28.99 ± 1.34 ^b

Values are given as mean ± SD (n = 3). Different letters (a-e) in the same column indicate significant difference (p ≤ 0.05). I: industrial raspberry seed oil (I1, I2, I3), L: laboratory-pressed raspberry seed oil (L1, L2, L3), T_c: peak temperature, h_c: peak height, ΔH_c: enthalpy.

flaxseed oil, a heating rate of 5 °C/min appeared to give almost identical melting curves for five different varieties of flax seeds (Tomaszewska-Gras et al., 2021). Therefore, it is necessary to analyse oils samples by more than two heating rates during the DSC characterization study. Other DSC parameters measured in raspberry seed oil's melting curves are peak height (h_m) and enthalpy (ΔH_m). Generally, peak heights increase as the heating rate increases. Meanwhile, the relationship between enthalpy and scanning rates is not so obvious to define,

Table 3

DSC parameters of melting curves for raspberry seed oil at scanning rates 1, 2, 5 °C/min.

Oil	Peak temperature (°C)			Peak height (W/g)			Enthalpy (J/g)		
	T _{m1}	T _{m2}	T _{m3}	h _{m1}	h _{m2}	h _{m3}	ΔH _{m1}	ΔH _{m2}	ΔH _{m3}
Heating rate 1 °C/min									
I1	-39.67 ± 0.13 ^c	-36.04 ± 0.02 ^b	-17.85 ± 0.04 ^{ab}	0.03 ± 0.002 ^a	-0.12 ± 0.006 ^e	0.23 ± 0.01 ^b	13.58 ± 2.68 ^a	-17.41 ± 0.03 ^c	75.12 ± 0.79 ^b
I2	-34.00 ± 0.00 ^d	-31.88 ± 0.86 ^c	-15.91 ± 0.13 ^b	0.06 ± 0.005 ^b	-0.16 ± 0.004 ^d	0.14 ± 0.00 ^a	22.29 ± 2.05 ^a	-23.15 ± 0.39 ^b	63.95 ± 1.50 ^a
I3	-41.15 ± 0.25 ^{ab}	-39.14 ± 0.25 ^a	-21.97 ± 0.24 ^{ab}	0.09 ± 0.006 ^c	-0.37 ± 0.006 ^a	0.25 ± 0.00 ^c	25.85 ± 4.41 ^c	-31.30 ± 0.72 ^a	71.91 ± 0.98 ^b
L1	-41.02 ± 0.01 ^b	-38.79 ± 0.04 ^a	-21.50 ± 0.08 ^{ab}	0.07 ± 0.000 ^b	-0.26 ± 0.006 ^b	0.27 ± 0.00 ^d	20.81 ± 1.73 ^a	-23.96 ± 1.46 ^b	65.48 ± 0.11 ^a
L2	-41.16 ± 0.19 ^{ab}	-38.92 ± 0.23 ^a	-21.76 ± 0.33 ^{ab}	0.09 ± 0.003 ^c	-0.27 ± 0.009 ^b	0.25 ± 0.00 ^c	38.32 ± 6.43 ^{bc}	-24.01 ± 1.21 ^b	76.02 ± 2.02 ^b
L3	-41.60 ± 0.01 ^a	-39.19 ± 0.01 ^a	-24.22 ± 4.89 ^a	0.07 ± 0.003 ^b	-0.20 ± 0.004 ^c	0.31 ± 0.00 ^e	44.23 ± 3.11 ^{ab}	-16.13 ± 0.45 ^c	90.55 ± 0.33 ^c
	T _{m1}	T _{m2}	T _{m3}	h _{m1}	h _{m2}	h _{m3}	ΔH _{m1}	ΔH _{m2}	ΔH _{m3}
Heating rate 2 °C/min									
I1	-37.30 ± 0.54 ^b	-34.14 ± 0.18 ^b	-17.51 ± 0.43 ^b	0.08 ± 0.01 ^a	-0.42 ± 0.03 ^a	0.37 ± 0.00 ^b	14.02 ± 0.71 ^a	-29.68 ± 1.00 ^a	69.93 ± 0.08 ^b
I2	-33.40 ± 0.35 ^c	-28.80 ± 0.35 ^c	-15.51 ± 1.20 ^b	0.12 ± 0.03 ^a	-0.05 ± 0.09 ^b	0.14 ± 0.04 ^a	24.00 ± 5.09 ^{ab}	-7.22 ± 8.87 ^b	31.18 ± 9.50 ^a
I3	-40.21 ± 0.01 ^a	-36.52 ± 0.01 ^a	-21.24 ± 0.13 ^a	0.27 ± 0.01 ^c	-0.54 ± 0.01 ^a	0.50 ± 0.00 ^c	45.71 ± 6.21 ^c	-42.46 ± 1.43 ^a	66.19 ± 1.26 ^b
L1	-39.69 ± 0.02 ^a	-37.13 ± 1.20 ^a	-20.94 ± 0.01 ^a	0.22 ± 0.01 ^{bc}	-0.51 ± 0.02 ^a	0.53 ± 0.00 ^c	43.94 ± 3.20 ^c	-34.73 ± 1.02 ^a	64.17 ± 0.30 ^b
L2	-40.22 ± 0.03 ^a	-36.86 ± 0.10 ^a	-21.46 ± 0.14 ^a	0.22 ± 0.01 ^b	-0.51 ± 0.02 ^a	0.50 ± 0.02 ^c	52.81 ± 2.71 ^c	-34.01 ± 0.31 ^a	68.39 ± 1.77 ^b
L3	-39.83 ± 0.14 ^a	-37.23 ± 0.04 ^a	-20.30 ± 0.26 ^a	0.19 ± 0.01 ^b	-0.57 ± 0.01 ^a	0.56 ± 0.01 ^c	36.48 ± 7.12 ^{bc}	-30.11 ± 0.06 ^a	74.58 ± 0.10 ^b
	T _{m1}	T _{m2}	T _{m3}	h _{m1}	h _{m2}	h _{m3}	ΔH _{m1}	ΔH _{m2}	ΔH _{m3}
Heating rate 5 °C/min									
I1	-33.68 ± 0.30 ^b	-24.84 ± 0.48 ^c	-15.26 ± 0.59 ^{bc}	0.38 ± 0.01 ^b	0.08 ± 0.02 ^d	0.14 ± 0.02 ^a	38.33 ± 0.541 ^b	7.77 ± 1.91 ^d	12.63 ± 1.724 ^b
I2	-37.34 ± 0.26 ^a	-30.60 ± 0.02 ^{ab}	-12.47 ± 0.20 ^c	0.15 ± 0.00 ^a	0.31 ± 0.03 ^c	0.03 ± 0.01 ^a	13.77 ± 0.718 ^a	35.51 ± 3.055 ^e	3.50 ± 0.107 ^a
I3	-36.89 ± 1.33 ^a	-27.82 ± 1.48 ^{bc}	-19.19 ± 1.87 ^a	0.64 ± 0.05 ^d	-0.19 ± 0.03 ^c	0.49 ± 0.01 ^b	42.09 ± 1.095 ^b	-13.39 ± 2.452 ^c	18.95 ± 1.727 ^c
L1	-38.56 ± 0.05 ^a	-30.64 ± 0.04 ^{ab}	-20.07 ± 0.11 ^a	0.55 ± 0.01 ^{cd}	-0.39 ± 0.00 ^b	0.87 ± 0.04 ^c	38.96 ± 0.160 ^b	-25.66 ± 0.02 ^b	36.15 ± 0.780 ^d
L2	-37.51 ± 0.01 ^a	-28.73 ± 0.22 ^{abc}	-19.95 ± 0.08 ^a	0.52 ± 0.01 ^c	-0.18 ± 0.02 ^c	0.45 ± 0.02 ^b	41.16 ± 0.460 ^b	-11.12 ± 1.09 ^c	19.58 ± 1.310 ^c
L3	-36.94 ± 0.24 ^a	-32.71 ± 2.47 ^a	-18.00 ± 0.32 ^{ab}	0.52 ± 0.02 ^c	-0.63 ± 0.02 ^a	1.01 ± 0.05 ^d	39.14 ± 1.770 ^b	-35.72 ± 0.20 ^a	52.83 ± 1.940 ^e

Values are given as mean ± SD (n = 3). Different letters (a-e) in the same column at the same scanning rate indicate significant difference (p ≤ 0.05). I: industrial raspberry seed oil (I1, I2, I3), L: laboratory-pressed raspberry seed oil (L1, L2, L3), T_m: peak temperature, h_m: peak height, ΔH_m: enthalpy,

because the general shape of melting curves changed completely in response to higher heating rates, which could be caused by polymorphic changes (Fig. 3E). Concerning the peak height and enthalpy, significant differences between the I1, I2 and rest of the samples were observed for the h_{m1} determined at all heating rates (p ≤ 0.05). However, the enthalpy was the DSC parameter that can be least utilized for a differentiation study of raspberry seed oil.

The crystallization and melting curves showed that samples containing more saturated fatty acids (I1, I2) were crystallized and melted at higher temperatures. The influence of fatty acid composition distribution was previously reported by Tomaszewska-Gras et al. (2021). As significant differences among samples were previously reported in fatty acid composition, the resulting curves could be used for qualitative differentiation study between oil samples.

3.5. Relationship between DSC parameters and fatty acid composition by PCA

Based on the melting and crystallization phase transition of raspberry seed oil, principal component analysis (PCA) was conducted to analyse the relationship between DSC parameters and fatty acid composition (Fig. 4). Heating rates 1 and 2 °C/min were chosen for PCA due to higher sensitivity of lower scanning rate to detect any changes in oils. Loading and scatter plots of PC1 (67.65 %) and PC2 (15.61 %) of

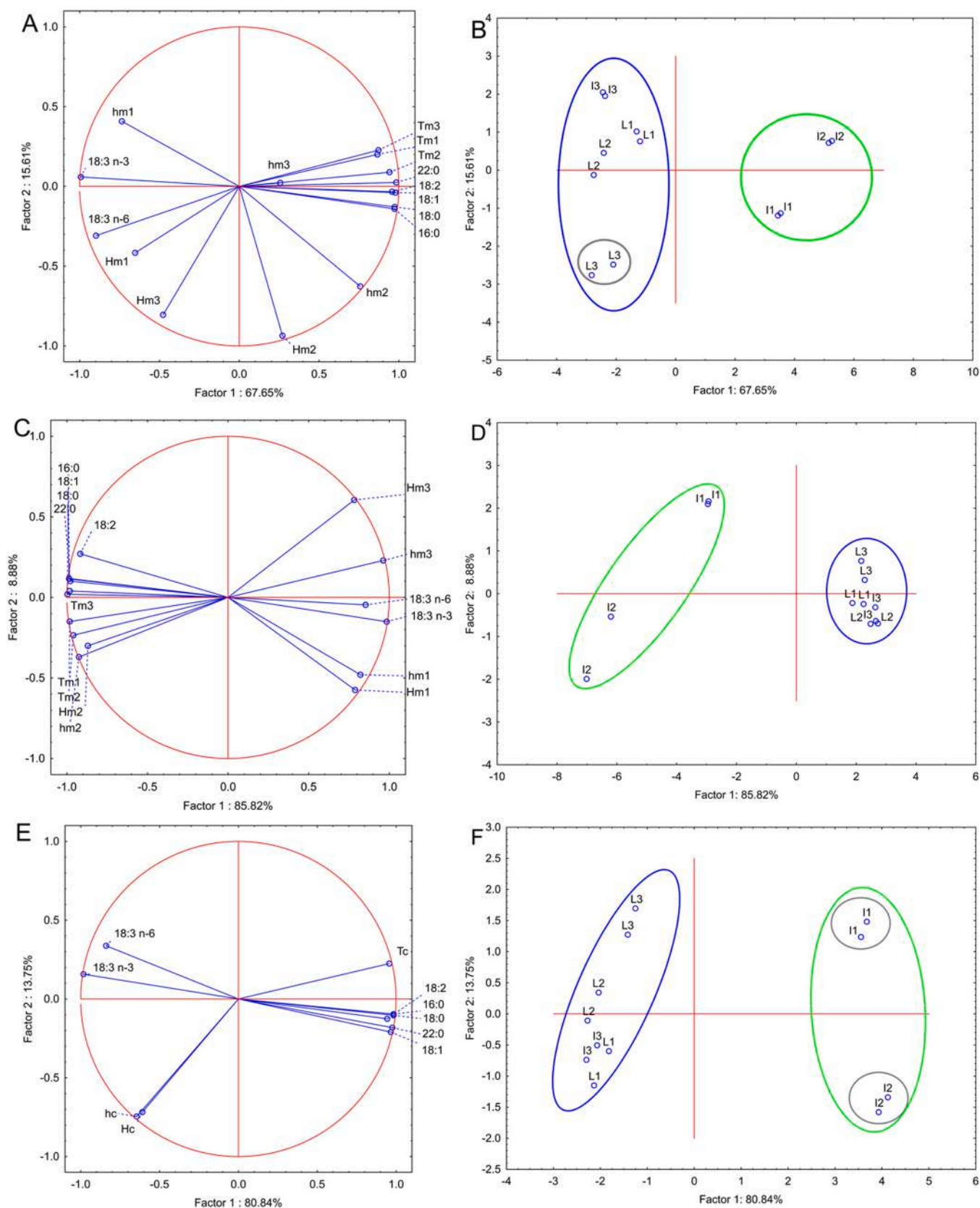


Fig. 4. The loading and scatter plots for PC1 and PC2 of DSC parameters and fatty acid composition of industrial and laboratory-pressed raspberry seed oils. PCA of DSC melting parameters at scanning rate 1 °C/min (A, B) at scanning rate 2 °C/min (C, D) DSC crystallization parameters at scanning rate 1 °C/min (E, F). I: industrial-pressed raspberry seed oil (I1, I2, I3), L: laboratory-pressed raspberry seed oil (L1, L2, L3), T_m: peak temperature, h_m: peak height, H_m: enthalpy.

heating rate 1 °C/min are depicted in Figs. 4A and 4B. The analysis showed an inverse relationship between T_m and triunsaturated fatty acids (18:3n-3 and 18:3n-6). Negative correlations between polyunsaturated fatty acids and T_m were previously highlighted in other studies (Tan and Che Man, 2000; Tomaszewska-Gras et al., 2021). For the rest of fatty acids, significant positive correlations with T_{m1} , T_{m2} , T_{m3} were observed ($p \leq 0.05$). A similar relationship was also shown in PCA with results of melting phase transition at a heating rate of 2 °C/min (Figs. 4C and 4D) and crystallization phase transition at a cooling rate of 1 °C/min (Figs. 4E and 4F). For all scanning rates, oil samples were divided into two clusters by PCA analysis, as can be seen on scatter plots (Figs. 4B, 4D, and 4F). The first cluster consisted of sample I1 and I2, while the rest of the samples belonged to the second cluster (Figs. 4B, 4D, and 4F). The results shown on loadings plots (Figs. 4A, 4C, 4E) indicate that, regardless of the scanning rate, there was always a significant negative correlation between triunsaturated fatty acids (18:3n-3 and 18:3n-6) and DSC parameters of peak temperatures. This is an important finding, since the shape of the DSC profile changed with the scanning rate, due to the ability of the triacylglycerols to undergo polymorphic transitions during melting, especially at lower heating rates. The presence of an exothermic peak for each heating rate proved a polymorphic transition. The relationships observed by PCA indicate that polyunsaturated fatty acids play a key role in shaping the DSC curve, and the variability of their amount is the cause of significant differences in the DSC profile between oils I1-I2 and other oils (L1-L3, I3). The results show DSC feasibility as a rapid characterization and authenticity tool to distinguish between two groups of raspberry seed oils (I- and L-group). On the other hand, it should also be highlighted that DSC has limitations as the sole technique to provide compositional information about oil.

4. Conclusions

Despite being extracted with the same method, cold-pressed raspberry seed oils from the I- and L-group were distinguishable based on its compositional, spectroscopic and thermal characteristics. Regardless of the variability in fatty acid composition, raspberry seed oils were characterized by a high nutritional quality, linked to cardioprotective property, as indicated by low AI, IT indices and high HH ratio. Generally, the analyses carried out enabled the samples to be distinguished into two clusters of L- and I-group oils, which is the result of a variation in fatty acid composition, UV/Vis and FTIR spectra, as well as DSC phase transition profiles. UV/VIS spectra revealed differences between oils samples in terms of presence of conjugated dienes and trienes, tocopherols and phenolic acids as well as carotenoids and chlorophylls compounds. FTIR spectra enabled to detect differences in ratio of UFA/SFA ratio, confirmed by the fatty acids analysis. Moreover, it was shown that FTIR can be used for differentiating oils in terms of quality based on the band assigned to free fatty acids presence, which was confirmed by the measurement of acid value. DSC technique was able to indicate thermal behaviour variability among samples of L-and I-group, regardless of the scanning rate used (1, 2, 5 °C/min). The melting curves at a heating rate 1 and 2 °C/min were considered useful for differentiating the oils as there were clear differences between groups L and I. For the oils pressed in the laboratory (L-group), two endothermic peaks were detected ($T_{m1} = -41$ °C, $T_{m3} = -22$ °C) and one exothermic peak ($T_{m2} = -39$ °C) at the scanning rate 1 °C/min, while for some I-group oils significantly higher values were observed ($p \leq 0.05$). Additionally, higher scanning rates resulted in higher peak temperatures and peak heights. All the obtained results provide practical information on the usefulness of instrumental methods, such as DSC or FTIR, to assess the quality and authenticity of raspberry seed oil. This can be useful for the oil manufacturers and B2B industries, interested in fast and non-targeted methods.

CRedit authorship contribution statement

Yolanda Victoria Rajagukguk: Conceptualization, Methodology, Investigation, Data curation, Visualization, Formal analysis, Writing-Original draft preparation. **Mahbuba Islam:** Investigation. **Anna Grygier:** Investigation. **Jolanta Tomaszewska-Gras:** Conceptualization, Methodology, Resources, Investigation, Validation, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2023.105723.

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Original article

Physicochemical characteristics of cold-pressed blackcurrant-, strawberry-, and raspberry seed oils during storage and its influence on thermo-oxidative stability

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ABSTRACT

The storage characteristics of cold-pressed blackcurrant-, strawberry- and raspberry seed oils and the factors influencing thermo-oxidative stability during storage were determined. It was established that throughout one year there were no significant ($p \leq 0.05$) changes of thermo-oxidative stability (OIT at 120 and 140 °C) and fatty acid composition in all berry seed oils (BSO), despite a minor reduction in antioxidant activity (DPPH radical scavenging) and tocopherol content, and an increase in acid value, p-anisidine value, and K_{232} , K_{268} values. Pigment degradation became noticeable after 6 months, resulting from a decrease in chlorophyll and carotenoid content, and observed in changes of colour parameters (angle hue, chroma, ΔE). The study also showed that many compositional factors play an important role in maintaining the thermo-oxidative stability of berry seed oils at a constant level over a period of one year, among which the most significant parameters influencing OIT values were the content of fatty acid c18:1, tocopherols and carotenoids, as revealed by the multiple linear regression analysis (MLR).

1. Introduction

In 2020, European Union launched the European Green Deal, an initiative aimed at a sustainable and circular economy reaching climate neutrality by 2050. In food processing, circular economy can be fostered by the valorisation of by-products, such as extracting berry seed oils from berry juice by-products. Trends in the utilization of berry seed oils as dietary supplements were observed due to its nourishing properties, such as abundant amounts of *n*-6 and *n*-3 fatty acids and bioactive compounds [1]. Compared to other conventional edible oils, berry seed oils (BSO) offer some distinctive values, including the rare presence of γ -linolenic acid in blackcurrant oil, which can also be found in human milk [2], the abundant amount of γ -tocopherol in raspberry oil [3], and strawberry oil as a rich source of α -linolenic acid [4]. Regarding its potential application in food industry, several studies reported BSO utilization in blended frying oils to improve frying quality and stability [5,6]. For instance, 1 % of raspberry seed oils added to conventional

cooking oils (i.e. soybean, canola, sunflower seed, and corn oils) were reported to exhibit high onset and peak oxidation temperatures, as well as low peroxide (PV), p-anisidine (p-AnV), and polar compounds during deep frying at 180 °C [6]. BSO intended for direct consumption is preferably extracted by cold-pressing and is unrefined, which aims to preserve natural bioactive compounds and nutritional quality. Production of BSO faces many challenges amid its rising popularity. BSO requires careful handling and storage due to high polyunsaturated fatty acids (PUFA) content, which are prone to oxidative degradation. It is important to maintain the oil quality during storage, as prolonged degradation may lead to the formation of an off-flavour and the accumulation of harmful substances. The next challenge is shelf life determination based on BSO quality parameters. As such parameters in BSO are yet to be regulated, commercial BSO might be produced without any awareness to meet the minimum threshold of bioactive compound content that benefits shelf life prolongation. A recent storage study highlighted a linear relationship between the initial content of

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carotenoids, chlorophyll, and phenolics with thermo-oxidative stability in seed oil [7]. Additionally, a study by Li et al. [8] reported the role of minor components, such as tocochromanols and pigments on the oxidative stability of stripped-BSO in an accelerated storage condition. In this regard, conducting thermo-oxidative stability analysis is necessary for a storage study as it hints the capability of the remaining bioactive compounds to protect oils.

Conventional chemical methods for the assessment of oxidative stability of oils such as peroxide value (PV) are subject to the technician's precision, and the quality of both the chemicals and the tools. Moreover, large amounts of harmful chemicals are used in these methods, which produces toxic chemical wastes [9]. Another option for determining the oxidation stability of oils may be the instrumental technique of differential scanning calorimetry (DSC), which allows determining thermo-oxidative stability based on the oxidation induction time (OIT). Several studies have explored the possibility to improve thermo-oxidative stability of cold-pressed seed oils, for instance, roasting the berry seeds prior to extraction [10], blending a certain proportion of the seed oils [11], or adding natural antioxidant to the oil [12]. In addition to these efforts, recent studies also covered the changes of thermo-oxidative stability in cold-pressed seed oils during storage [9,13,14]. Thermo-oxidative stability changes are related to fatty acid composition, antioxidant content, storage conditions, presence of pro-oxidants, and refinement [15]. Despite these progressive efforts, the knowledge about BSO characteristics in relation to its thermo-oxidative stability is still limited.

Li et al. [8] reported the significance of tocochromanols content in BSO to protect the oils against high temperature and photo-induced oxidation in an accelerated storage that was equivalent to 7 months of observation. Following the attempt, Raczyk et al. [16] studied the changes of BSO during the 8 weeks of storage after the bottle was opened. Unfortunately, both existing studies provided limited insight into the actual store-like conditions before it reach the consumers' hands. To the best of our knowledge, presented work is the first attempt to study the characteristics of blackcurrant-, raspberry- and strawberry seed oils during storage in store-like conditions with an emphasis on the thermo-oxidative stability.

The first aim of this study was to evaluate the physicochemical and compositional characteristics of BSO during one year of storage in store-like conditions with an emphasis on the relationship among quality parameters. Secondly, it aimed to determine the factors influencing the thermo-oxidative stability of BSO during storage. The knowledge provided in this study might benefit BSO producers in determining the shelf life of raspberry-, blackcurrant- and strawberry seed oils, as well as in optimising the general BSO production.

2. Material and methods

2.1. Materials

Three types of cold-pressed berry seed oils, namely blackcurrant (*Ribes nigrum*), strawberry (*Fragaria ananassa*), and raspberry (*Rubus idaeus*), obtained from three different production batches, were purchased from two different suppliers: GreenField Sp. z.o.o (Warsaw, Poland) and Olvita Gotuch Sp. k. (Marcinowice, Poland). Initial physicochemical and compositional characteristics of these samples were compared to the oils that were freshly-pressed in the laboratory to ensure its authenticity. The oils were then kept in a brown-glass bottle without headspace at $\pm 20^\circ\text{C}$ for one year to mimic the storage conditions in a regular oil shop. The observations were conducted at 0, 3, 6, 9, and 12 months of storage. In total, 45 oil samples (15 samples from each type of oil) were analysed throughout the storage observation.

2.2. Physicochemical analysis

The storage characteristics of oils were analysed based on

physicochemical properties with the following criteria being analysed: acid value (AV), p-anisidine value (p-AnV), specific absorbance coefficients (K-values), colour, and DPPH radical scavenging activity. The physicochemical properties of each oil was determined in duplicate for each oil sample.

2.2.1. Acid value

Acid value (AV) was determined following the standard ISO 660 [17]. The obtained value was expressed as mg KOH g/fat, indicating the amount of KOH needed for free fatty acid neutralization in one gram of fat.

2.2.2. Determination of p-anisidine value

The p-anisidine value (p-AnV) was determined based on the ISO 6885 method [18]. The analysis determined the amount of secondary oxidation products in the oil, which correlates with rancidity. The secondary oxidation products reacted with p-anisidine in acetic acid environment, which resulted in changes in absorbance at 350 nm.

2.2.3. Specific absorbance coefficient of oils (K-values)

K-values were determined following the method described by AOCS ch5-91 [19]. The values indicate the presence of conjugated dienes (K_{232}) and conjugated trienes (K_{268}) formed as oxidation products in the oil.

2.2.4. Colour analysis

For colour analysis a Konica Minolta CM-5 spectrophotometer (Konica Minolta, Inc., Tokyo, Japan) was used. The oils' colour characteristics were determined based on the CIELAB colour system (L^* , a^* , b^* , C^*_{ab} , and h_{ab}). L^* (1–100) indicated the range of brightness from black to white. Parameters a^* and b^* indicated the traces of green-red and blue-yellow colours, respectively. In this study, colour characteristics during storage were presented as the chroma (C^*_{ab}), angle hue (h_{ab}), and ΔE , which were calculated based on the following eqs.

$$C^*_{ab} = \sqrt{(a^*)^2 + (b^*)^2} \quad (1)$$

$$h_{ab} = \tan^{-1}(b^*/a^*) \quad (2)$$

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (3)$$

Chroma indicates the relative saturation, angle hue indicates the gradation of colour based on the colour wheel's angle, while ΔE quantifies the difference between the oil's initial colour (0 month) and in subsequent months (3, 6, 9, 12 months) of storage.

2.2.5. DPPH radical scavenging activity

DPPH radical scavenging activity was determined based on the method described by Sánchez-Moreno et al. [20]. Oil diluted in ethyl acetate (100 μL) was mixed with a DPPH radical solution (1 mmol/L, 250 μL) and ethyl acetate (2 mL). The sample mixture was then mixed thoroughly and kept in the dark for 20 min. The absorbance reading was conducted at 515 nm. DPPH radical scavenging activity was expressed as μmol of Trolox equivalent (TE) per gram of oil.

2.3. Compositional analysis

Several compositional properties analysed in this study are total content of carotenoids, chlorophyll, tocopherols, and fatty acids. The content of carotenoids and chlorophyll was investigated at 0, 3, 6, 9, and 12 months of storage, while both total tocopherol and fatty acid content were investigated at the beginning (0 month) and at the end (12 month) of storage. Oil composition was determined in duplicate for each oil sample.

2.3.1. Total carotenoid content

Total carotenoid content in the berry seed oils was measured following the method described by Romanić et al. [21]. Berry seed oil (0.2–1 g) was mixed thoroughly with 10 mL of cyclohexane. The absorbance was measured at 445 nm and it should range between 0.1 and 0.8. Total carotenoid was expressed as mg of β -carotene/kg of oil, calculated as follows:

$$\text{Total carotenoid } (\beta - \text{carotene}) = \frac{A}{m \times 100} \times 383 \quad (4)$$

A is the absorbance at 445 nm, while m is the sample mass.

2.3.2. Chlorophyll content

The chlorophyll content in the berry seed oil was determined following the method described by AOCS cc13i-96 [22]. Berry seed oil (0.2–1 g) was mixed thoroughly with 10 mL of isooctane. If the sample had impurities, it was filtered prior to the absorbance reading with filter paper of medium porosity. Absorbance measurements were conducted at 630 nm, 670 nm, and 710 nm against air, not a reference cell. Chlorophyll content was calculated as follows:

$$\text{Chlorophyll pigments} = 345.3 \times \frac{(A_{670} - (0.5 \times A_{630}) - (0.5 \times A_{710}))}{L} \quad (5)$$

A is the absorbance at the respective wavelengths and L is the light path of the spectrophotometer cell (mm). The chlorophyll pigment content was expressed as mg of pheophytin a.

2.3.3. Total tocopherol content

The tocopherol content of the berry seed oils was determined based on the method described by Siger et al. [23]. The oil (200 mg) was dissolved in n-hexane (10 mL) and mixed thoroughly in a volumetric flask. Prior to the analysis, the mixture was transferred into a vial. Tocopherol content was determined using a Waters HPLC system (Waters, Milford, MA) equipped with a pump (Waters 600), fluorometric detector (Waters 474), photodiode array detector (Waters 2998 PDA), autosampler (Waters 2707), column oven (Waters Jetstream 2 Plus), and LiChrosorb Si 60 column (250 $\times$ 4.6 mm, 5 μ m) by Merck (Darmstadt, Germany). Standards (α -, β -, γ -, and δ -tocopherols) with >95 % of purity were purchased from Merck (Darmstadt, Germany). The mobile phase consisted of n-hexane and 1,4-dioxane (96:4 v/v) ran at 1 mL/min. The excitation and emission of fluorescence from tocopherol were recorded at 295 nm.

2.3.4. Determination of fatty acids content

Fatty acids content was determined using gas chromatography (GC), according to AOCS [24]. An internal standard was prepared by dissolving 0.125 g of methyl heptadecanoate (Sigma Aldrich, USA) in 25 mL of hexane, from which 100 μ L was added to the oil sample (± 0.01 g). This mixture was then dissolved in 1 mL of hexane (>99 %, Avantor, Gliwice, Poland) and 1 mL of 0.4 M sodium methoxide (Merck, Darmstadt, Germany). After being properly mixed, the mixtures were kept still for 15 min. Afterwards, 5 mL of distilled water was added into the mixture to stop the reaction. The upper layer was taken for GC-analysis. Gas chromatography (Trace 1300) with a FID detector (Thermo Scientific, Waltham, MA, USA) was equipped with a capillary column SP TM-2650 with a dimension size of 100 m $\times$ 0.25 mm $\times$ 0.2 μ m (Supelco, Bellefonte, PA, USA). Hydrogen gas (1.5 mL/min) was used as the carrier in the splitless mode of analysis. Analysis was carried out by heating the oven to 160 $^{\circ}$ C for 1 min, then the heat was raised to 220 $^{\circ}$ C by 6 $^{\circ}$ C/min, while the temperature of inlet and detector was set at 240 $^{\circ}$ C. By comparing the sample's retention time with the reference material (37 Component FAME Mix, Supelco), the content of some prominent fatty acids in the sample was able to be determined. The content of fatty acids was expressed in mg/g sample.

2.4. Thermo-oxidative stability

Isothermal analysis by differential scanning calorimetry (DSC) was carried out following the method described in ISO 11357-6 [25] with a slight modification for berry seed oils [3]. Perkin Elmer DSC 7 (Perkin Elmer, Norwalk) with Intra cooler II and Pyris software were utilised. Nitrogen with 99.99 % purity was used as the purge gas. The machine was calibrated using indium (m.p. 156.6 $^{\circ}$ C, ΔH_f = 28.45 J/g) and n-dodecane (m.p. - 9.65 $^{\circ}$ C, ΔH_f = 216.73 J/g). An open aluminium pan (50 μ L, Perkin Elmer, No. 02190041) was filled with oil (6–7 mg). During the analysis, samples were under the constant exposure of oxygen (99.995 % purity) at 20 mL/min flow. The heat differences of the sample were compared with the empty open-aluminium pan. Isothermal analysis was conducted to determine the thermo-oxidative stability of oils based on oxidative induction time (OIT) at 120 and 140 $^{\circ}$ C.

2.5. Data analysis

The data were presented in the form of means calculated from three different batches of each BSO. Principal component analysis (PCA) was carried out to explore the distribution of the input variables, while Pearson's correlation analysis was performed to evaluate the relationship between all parameters. Multiple linear regression (MLR) analysis was employed to identify key variables and to predict the thermo-oxidative stability of oils measured by oxidation induction time (OIT). For this purpose, all compositional and physicochemical parameters were used as independent variables (predictors) to build predictive models, in which the dependent variable was the oxidation induction time (OIT) measured at 120 and 140 $^{\circ}$ C. Due to the large set of variables, a hierarchical regression approach with forward stepwise regression was used, in which algorithms determined which predictors enter the model by successively introducing significant predictors until a statistical stopping criterion is reached. The goodness of fit of the regression models was estimated based on the coefficient of determination (R^2). All statistical analyses were performed using Tibco Statistica 13.3 software (Tibco Software Inc., Tulsa, OK, USA). Visualisation was conducted using OriginPro 10.1 software (OriginLab Corporation, Northampton, MA, USA).

3. Results and discussion

3.1. Storage characteristics of berry seed oils

Storage characteristics of BSO were examined based on the physicochemical and compositional properties during one year of observation. Physicochemical properties (Fig. 1) included the acid value (AV), p-anisidine value (p-AnV), K_{232} - K_{268} values, DPPH radical scavenging activity, content of bioactive compounds (carotenoids, chlorophyll), colour (chroma, angle hue, and ΔE), and thermo-oxidative stability, expressed as oxidative induction time (OIT). Additionally, content of the fatty acids and tocopherols of berry seed oils was determined, as depicted in Fig. 2.

3.1.1. Physicochemical characteristics of oils during storage

The physicochemical characteristics of BSO, shown in Fig. 1 represent the average value of each oils calculated from three different production batches. The acid values (AV) of all oils throughout storage are presented in Fig. 1A. At the beginning of storage, AV of each oils averaged 1.90, 3.68, and 7.15 mg KOH/g oil in blackcurrant-, strawberry-, and raspberry oil, respectively. According to CODEX quality standard for cold-pressed oil, AV in raspberry oil exceeded the limit at 4.0 mg KOH/g oil [26]. The reported AV in this study is slightly higher than in other studies, which are in the range of 1.74–3.45 mg KOH/g oil for raspberry oil [4,16]. Nevertheless, it is still lower than the AV of raspberry oils extracted using n-hexane [27]. In contrast, the initial AV of strawberry and blackcurrant were below the CODEX limit. These values were in

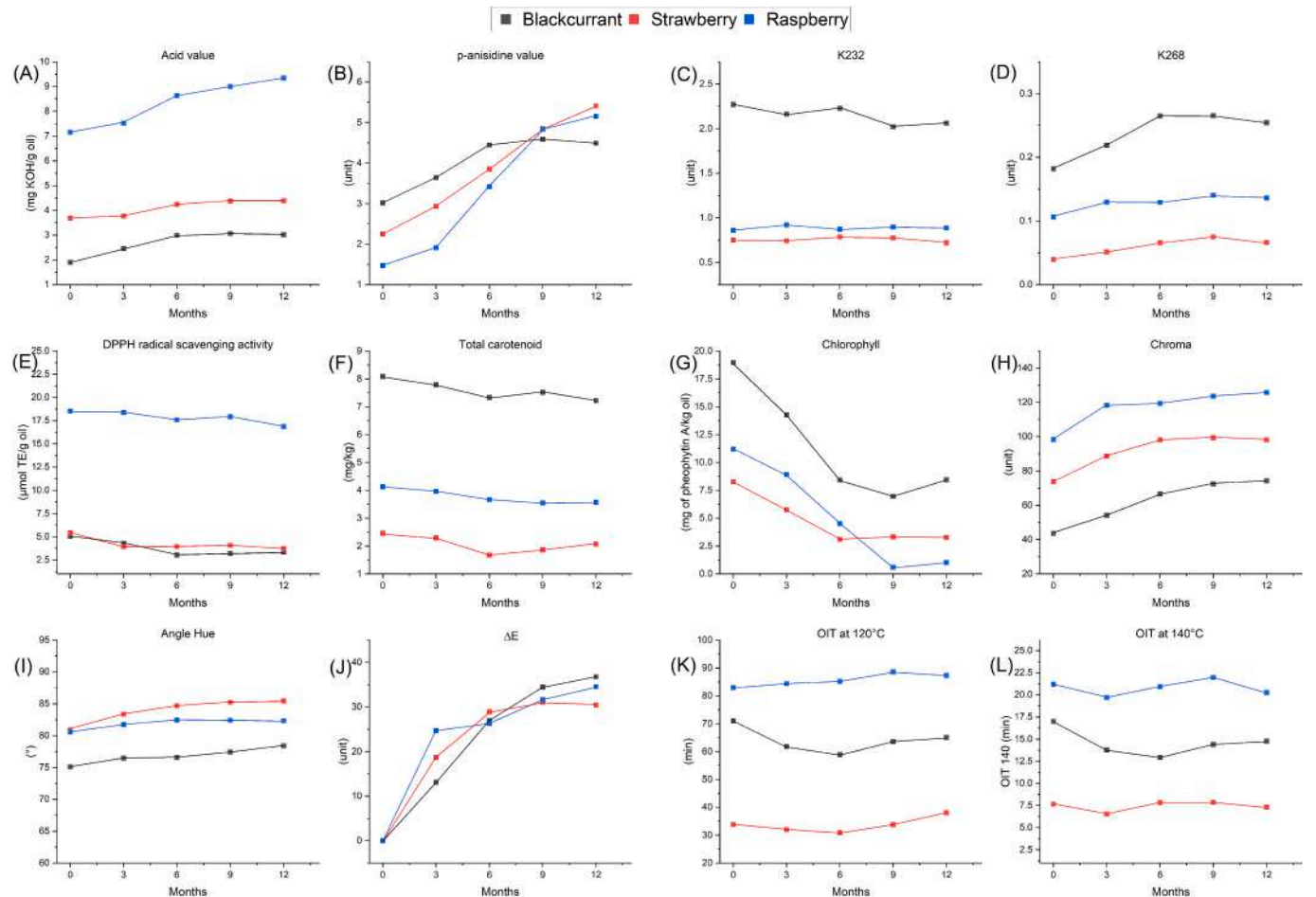


Fig. 1. Physicochemical characteristics and thermo-oxidative stability changes of berry seed oils throughout one year of storage.

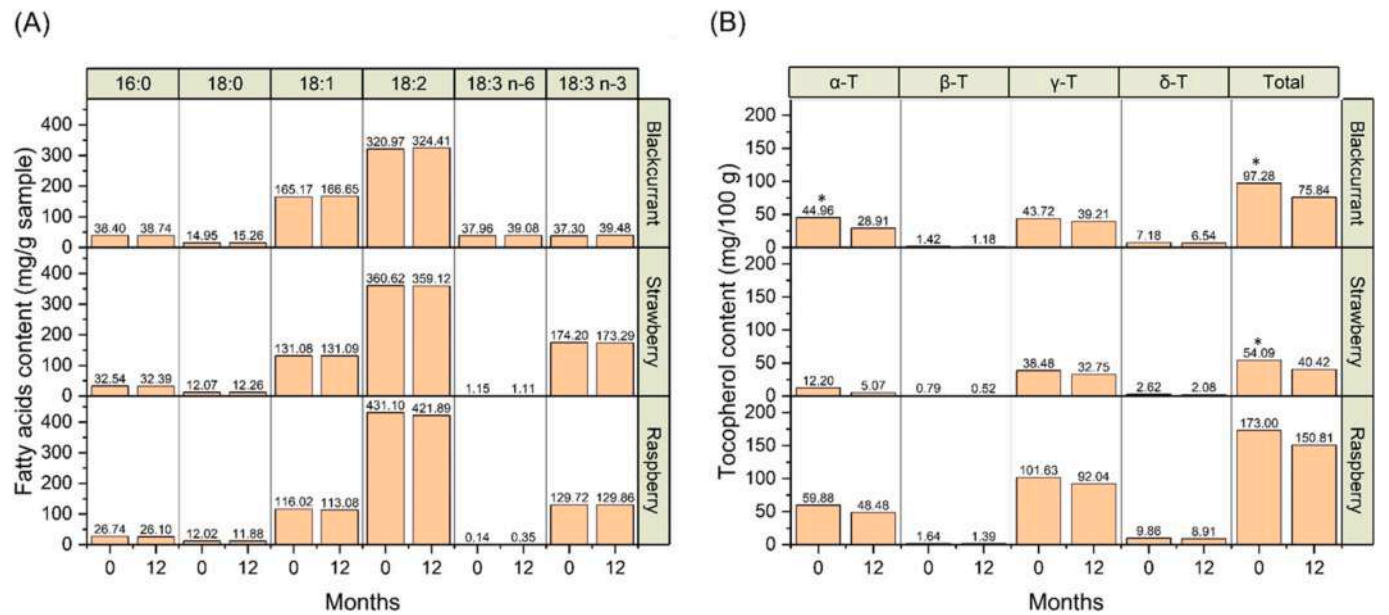


Fig. 2. (A) Changes of fatty acid content (mg/g) and (B) tocopherol content (mg/100 g) throughout one year of storage of berry seed oils; α-T: α-tocopherol; β-T: β-tocopherols; γ-T: γ-tocopherols; δ-T: δ-tocopherol. Bars marked with (*) indicate statistically significant values observed within the same oil and compound type ($p \leq 0.05$).

agreement with the literature, which are in the range of 2.09–3.02 mg KOH/g oil for strawberry oil and 4.10 mg KOH/g oil for blackcurrant oil [4,16]. In strawberry oil, AV exceeded the CODEX quality standard after 6 months, while blackcurrant oil remained below the limit during one year of storage. The values in all oils increased moderately, indicating the occurrence of hydrolytic reaction due to the initial oil condition throughout storage.

Acid value (AV), expressed as the acidity (%), is one of the most important quality parameters for olive oil categorisation, which is included in the European Commission Regulation (Reg. EU 2022/2104) [28]. It represents the raw material quality and its treatment prior to oil extraction. The wide range of AV in berry seed oils at the 0 months may indicate different methods and periods of seed processing. It should be taken into account that the seeds are collected as waste after fruit juice pressing and must be properly protected against spoilage. After berries have been processed into juice or jam, several possible pre-treatments, such as drying, mechanical separation from pomace, freezing and thawing, might be used before the oil is extracted [29]. In this regard, the raw material of berry seed oils may take longer processing steps and pre-treatments than any other ready-to-press oil seeds, as regulated by CODEX. Additionally, exposing the seeds to various temperatures was proven to cause variability in the berry oil's acidity [30].

Another parameter useful for assessing oil quality, p-anisidine value (p-AnV) determines the quantity of secondary oxidation products such as 2-alkenals and 2,4-alkadienals in oil during storage. The values of p-AnV increased during 12 months of storage for all oils, as presented in Fig. 1B, i.e. from 3.00 to 4.60 for blackcurrant, from 2.24 to 5.41 for strawberry, and from 1.46 to 5.15 for raspberry oil. The values for all months were lower than the reported in the previous studies [15,29,31]. The p-AnV at 0 months indicated the oxidation history during pre-treatment, while the p-AnV from 3 to 12 months represented the berry seed oils stability during oxidative propagation stage. In contrast to the AV value, the lowest values of p-AnV at 0 months were observed for raspberry seed oil and the highest for blackcurrant seed oil. At the end of storage, after 12 months, the highest values were obtained for strawberry seed oil and the lowest for blackcurrant seed oil.

Specific extinctions at 232 and 268 nm or K-values, as shown in Fig. 1C and D, are often used as a rapid tool for assessing oil quality in edible oils. The value of K_{232} corresponds to the amount of primary oxidation products in the form of conjugated dienes, while K_{268} signifies the secondary oxidation products, such as conjugated trienes. In this study, K_{268} was used to complement p-AnV, as it provides a general measurement of secondary oxidation products. During 12 months of observation, K_{232} values decreased in blackcurrant oil, indicating hydroperoxide decomposition as it entered the secondary oxidation stage. The decrease in K_{232} values in blackcurrant oil was followed by an increase in the K_{268} value at the 6th month of observation. Meanwhile, K_{232} values in strawberry and raspberry oils barely changed over the storage time at a level twice lower than blackcurrant oil. The results were supported by K_{268} , which remained stable after 12 months of storage. Both initial K_{232} and K_{268} values in all oils meet the quality pass for virgin olive oil grade, which are $K_{232} \leq 2.60$ and $K_{268} \leq 0.25$ [28]. It can be said that no major oxidative deterioration took place in bottled berry seed oils kept in store-like conditions for one year.

Antioxidant activity of oils was measured by DPPH radical scavenging activity as presented in Fig. 1E. The initial values were 5.04, 5.44, and 18.52 $\mu\text{mol TE/g oil}$ for blackcurrant, strawberry, and raspberry seed oil, respectively. A slight decrease in DPPH radical scavenging activity was observed during one year of storage. The results were followed by negligible changes in total carotenoids in all oils during storage (Fig. 1F). Blackcurrant, strawberry, and raspberry oil contained 8.09, 2.46, and 4.13 mg/kg oil of total carotenoids, respectively, at the beginning of storage, and after 12 months these compounds decreased to 7.21 (11 %), 2.07 (16 %), and 3.57 (14 %) mg/kg oil, respectively. This relatively slight decline throughout storage illustrates the antioxidative role of carotenoids during autooxidation. Carotenoids

stabilize the oil by quenching singlet oxygen and scavenging free radicals, resulting to its degradation into epoxide or carbonyl derivative [32]. Additionally, it was reported that the presence of carotenoids in PUFA rich oils can maintain the oxidative stability of oil throughout storage [33].

Contrary to the negligible changes in total carotenoids, the chlorophyll content in all oils decreased rapidly after the 3rd month of storage (Fig. 1G). Chlorophyll content hit the lowest value after 9 months for raspberry oils, and 6 months for both strawberry and blackcurrant oils. Similar results were also reported in virgin olive oil, which lost 50 % of its chlorophyll content after only 6 months of storage [34]. After 12 months of storage, the reduction of chlorophyll was 56 % in blackcurrant-, 61 % in strawberry- and 91 % in raspberry seed oil. The factors affecting chlorophyll degradation were temperature, UV light, oxidation, presence of acids, and prolonged storage. The drop in chlorophyll content during storage was mainly caused by light-induced photo-degradation, which severely affected chlorophyll than carotenoid in edible oil [35]. As the chlorophyll concentrations decreased, the remaining chlorophyll may no longer act as an antioxidant nor metal chelating agent, but as a pro-oxidant in the presence of light by facilitating oxygen conversion to singlet oxygen [36].

Degradation of total carotenoids and chlorophyll content was followed by changes in chroma (Fig. 1H), angle hue (Fig. 1I), and ΔE (Fig. 1J) of all oils during storage. Chroma indicates the colour intensity compared to a grey colour, angle hue specifies the colour types (red, yellow, green, blue) in the colour wheel, and ΔE expresses the colour differences throughout storage. All oils became more vivid as chroma values gradually increased until the end of storage. In accordance with total carotenoid and chlorophyll content, blackcurrant oil possessed the most muted colour compared to strawberry and raspberry oils. The angle hue were in the range of 75.09–78.41, 80.92–85.46, and 80.55–82.33 ($^{\circ}$) for blackcurrant, strawberry, and raspberry oils. The respective values imply that the oils turn to vivid-yellowish after one year of storage. The value of ΔE increased rapidly until the 6th month of storage, followed by a gradual increase until the end of storage. In the 6th month of storage, ΔE in strawberry oils increased by 95.05 %, followed by blackcurrant at 73.28 % and raspberry at 76.17 %. Since colour changes are clearly visible within 12 months, which are mostly seen by the logarithmic increase in the ΔE values, it can be assumed that this is due to a decrease in the amount of chlorophyll. Besides this pigment degradation, ΔE values were also reported to be influenced by oxidation and formation of secondary oxidation products over time [32].

The parameter investigated in this study to express the thermo-oxidative stability of oils, examined by differential scanning calorimetry (DSC), was the oxidation induction time (OIT). BSO were exposed to a constant oxygen flow in isothermal conditions at 120 $^{\circ}\text{C}$ or 140 $^{\circ}\text{C}$. Oxidative induction time (OIT), as depicted in Fig. 1K and L, represents the period of time needed until the initiation process took place during heat-induced oxidation. A high OIT value means a high thermo-oxidative stability possessed by the sample. The initial OIT values at 120 $^{\circ}\text{C}$ of blackcurrant, strawberry, and raspberry oils were 71.01, 33.82, and 82.95 min, respectively. The values for raspberry and strawberry oils were two times higher than the commercial oils reported by Raczky et al. [16]. A large difference in oxidative stability raises the alert of a possible adulteration or deterioration in commercial berry seed oils sold on the market. Another possible reason behind the differences might be related to the refining process conducted by the producer to suit the regulation in-force, which unfortunately eliminated the natural antioxidants in berry oils.

When the oils were heated at an isothermal temperature of 140 $^{\circ}\text{C}$, the initial OIT values obtained were 16.98, 7.65, and 21.19 min for blackcurrant, strawberry, and raspberry oils, respectively. These values were within the range of OIT of berry seed oils extracted from roasted and unroasted seeds by Róžańska et al. [10]. Berry seed oils provide relatively good protection during thermo-oxidation thanks to natural

antioxidants such as tocopherol, carotenoids, and polyphenols that are present in the oil [15]. The initial OIT differences for fresh oils and final after 12 months at both temperatures for all oils were ± 5 min. As the OIT at 120 and 140 °C was barely changed, it can be said that the berry seed oils are highly stable throughout 12 months of storage.

Summing up, after one year of storage, the physicochemical characteristics of berry seed oils were marked by a colour change caused by the pigment degradation, mainly chlorophyll. The colour changes become noticeable after 6 months of storage. Minor oxidative deterioration took place during storage, which was mainly caused by the initial quality state of the oil. Despite the changes, the thermo-oxidative stability of berry seed oils remained stable.

3.1.2. Compositional characteristics during storage

The average values of fatty acids (FA) content in blackcurrant, strawberry and raspberry oils were obtained from three different production batches (Fig. 2A). All fresh berry seed oils contained a high amount of polyunsaturated fatty acid (PUFA), 396, 536 and 561 mg/g in blackcurrant, strawberry and raspberry respectively, which indicates its potential functionality as a health-promoting agent [37]. This beneficial composition of PUFA, constituting 65–78 % of all fatty acids, also means that these oils are more prone to degradation, leading to a decline in oxidative stability during storage [38]. However, despite the high PUFA content in BSO, there were no statistically significant changes in FA content throughout the storage ($p \leq 0.05$). Similar observations on cold-pressed hemp seed oil were presented by Tura et al. [14].

Fresh blackcurrant oil mainly consists of linoleic (c18:2 n-6) and oleic (c18:1) acids, which account for up to 51 % (320.97 mg/g) and 26 % (165.17 mg/g) of the composition, respectively. These values were higher compared to the values in the existing literature on cold-pressed blackcurrant oil, with 46 % linoleic acid content and 15 % oleic acid content [39]. The most distinguishable characteristics of blackcurrant oil is the presence of γ -linolenic acid (c18:3 n-6), which is rare in most plant oils [2]. In contrast to linoleic and oleic content, the average value of γ -linolenic acid from three different blackcurrant oils batches was 6 % (37.96 mg/g), a value that is 9 % below than in the existing literature [39]. Bakowska-Barczak et al. [40] reported a strong negative correlation between linoleic and γ -linolenic acid in five different varieties of blackcurrant oils. Hence, when blackcurrant oil contains a high proportion of linoleic acid, it has a low γ -linolenic acid content. The FA composition in strawberry oils was dominated in 50 % by linoleic (360.62 mg/g), in 24 % by α -linolenic, c18:3 n-3 (174.20 mg/g), and in 18 % by oleic acid (131.80 mg/g). Strawberry oils possessed the highest amount of α -linolenic fatty acid compared to blackcurrant (37.30 mg/g) and raspberry (129.72 mg/g) oils. A similar FA composition of cold-pressed strawberry oils was reported by Pieszka et al. [4]. On the other hand, raspberry oils contained the highest amount of linoleic acid, 431.10 mg/g (59 %) compared to the rest of the oils, followed by of α -linolenic (18 %) and of oleic acids (15 %). These values are within the similar range as FA content of cold-pressed raspberry oils reported in the literature [4,41].

The second important lipid component of oils, apart from FA, are tocopherols, the composition of which is shown in Fig. 2B. Contrary to the minor changes in FA content during one year of storage, gradual decreases in tocopherol content were observed in all oils (Fig. 2B). Each berry seed oil contained a different ratio of α -, β -, γ -, and δ -tocopherols. The amount of tocopherol in berry seed oils decreased in the following order: raspberry > blackcurrant > strawberry oil. At first glance, it can be seen that tocopherol contents corresponded to the OIT values (Fig. 1K and L), as oil with the highest tocopherol contents exhibited the highest thermo-oxidative stability. Berry seed oils are mainly dominated by γ -tocopherol, followed by α - and δ -tocopherol with a ratio of 10:6:1, 15:5:1, and 6:6:1 for raspberry, strawberry, and blackcurrant oils, respectively. After one year of storage, the total tocopherol reduction varied from 13 % (for raspberry seed oil) to 22 % (for blackcurrant) and 25 % (for strawberry seed oil). The biggest loss of α -tocopherol was

observed in strawberry oils, which decreased up to 58 % from its initial value, while in blackcurrant and raspberry oils the reduction was up to 35 % and 19 % from the initial values, respectively. One of the roles of α -tocopherol in the oil was to protect β -carotene from degradation. The loss of α -tocopherol content might be related to its synergistic effect with β -carotene against lipid peroxidation by decreasing autooxidation and photosensitized oxidation in oil [32]. Moreover, α -tocopherol was shown to be more vulnerable to degradation than γ -tocopherol during the storage of olive oil at ambient temperature [42]. A minor reduction in γ -tocopherol was observed within one year, standing at 15 % in strawberry, 10 % in blackcurrant, and 9 % in raspberry oils. The γ -tocopherol was reported to have a higher antioxidant activity than α -tocopherol. As the molecular structure of γ -tocopherol only contains two methyl groups attached to the chromanol ring, it requires a lower bond dissociation energy for hydrogen donation, which results in prominent antioxidative activities [43,44]. This might be the reason behind the high DPPH radical scavenging activity in raspberry oil (Fig. 1E), as it contains a higher level of γ -tocopherol than other oils. However, it should be noted that a higher γ -tocopherol content does not equally translate to higher DPPH radical scavenging activity, as similar values were reported in blackcurrant and strawberry oils. Zaunschirm et al. reported that antioxidative activity and oxidative stability of edible oil were the result of a synergistic relationships between the optimum ratio of ($\gamma + \delta$)/ α -tocopherols [45].

The decrease in total tocopherol content is followed by a moderate increase in lipid oxidation products expressed by values of K_{232} and K_{268} (Fig. 1C and D). Tocopherols in berry seed oils were reduced to some extent over one year, but it should be noted that the remaining tocopherols in unopened bottles were still relatively high and proved to be adequate for protecting the oil during heat-induced oxidation.

3.2. Relationships between quality indicators and thermo-oxidative stability of oils

3.2.1. Differentiating characteristics of berry seed oils

PCA was performed using the individual data from each oil type and batch (Fig. 3A). These data were reduced into three dimensions PC1, PC2 and PC3 that cover 81.2 % of total variances. The distribution of the three berry seed oils into three clusters is shown by the 3D score plot on the biplot (Fig. 3A). Data points from raspberry oils were mainly scattered around the variables such as tocopherol, DPPH, AV, p-AnV, c18:2, and ΔE . It indicates raspberry oils' distinctive characteristics in terms of thermo-oxidative stability and antioxidant activity, as well as its changes throughout storage, as observed in AV, p-AnV, and ΔE . Blackcurrant oils were scattered around variables such as carotenoid, chlorophyll, OIT, K-values, and c18:1, confirming its distinctive features in bioactive compounds content, fatty acid, and the presence of oxidation products. Strawberry oils were at the lower part of the 3D score plot. The position explains the distinctive colour characteristics (chroma, angle hue) and fatty acid composition (c18:3 n-3) of strawberry oils.

The relationship between each variables with PCs is shown in Fig. 4. PC1, with 32.0 % of variance, correlates positively with thermo-oxidative stability (OIT 120, 140), antioxidant activity (DPPH, tocopherol), fatty acids (c18:3 n-3, c18:2), bioactive compound (chlorophyll, carotenoid), AV, and p-AnV (Fig. 4A). The loading plot in Fig. 4a also confirmed the negative correlation of PC1 with colour changes (angle hue, ΔE) and the presence of other oxidation products (K_{232} , K_{268}). The PC2 covers 27.1 % of total variance (Fig. 4B) and parameters such as colour (ΔE , angle hue, chroma), thermo-oxidative stability, and antioxidant activity were positively contributed to its variance. The inverse relationship between colour characteristics (ΔE , angle hue, chroma) and bioactive compounds content (chlorophyll, carotenoid) was established, demonstrating that PC2 explains colour changes in oils during storage, caused by a reduction in chlorophyll and carotenoids content. In turn, PC3 (Fig. 4c), explaining 22.1 % of variance, was positively correlated with OIT 120, OIT140 and negatively with α -linolenic fatty acid (c18:3

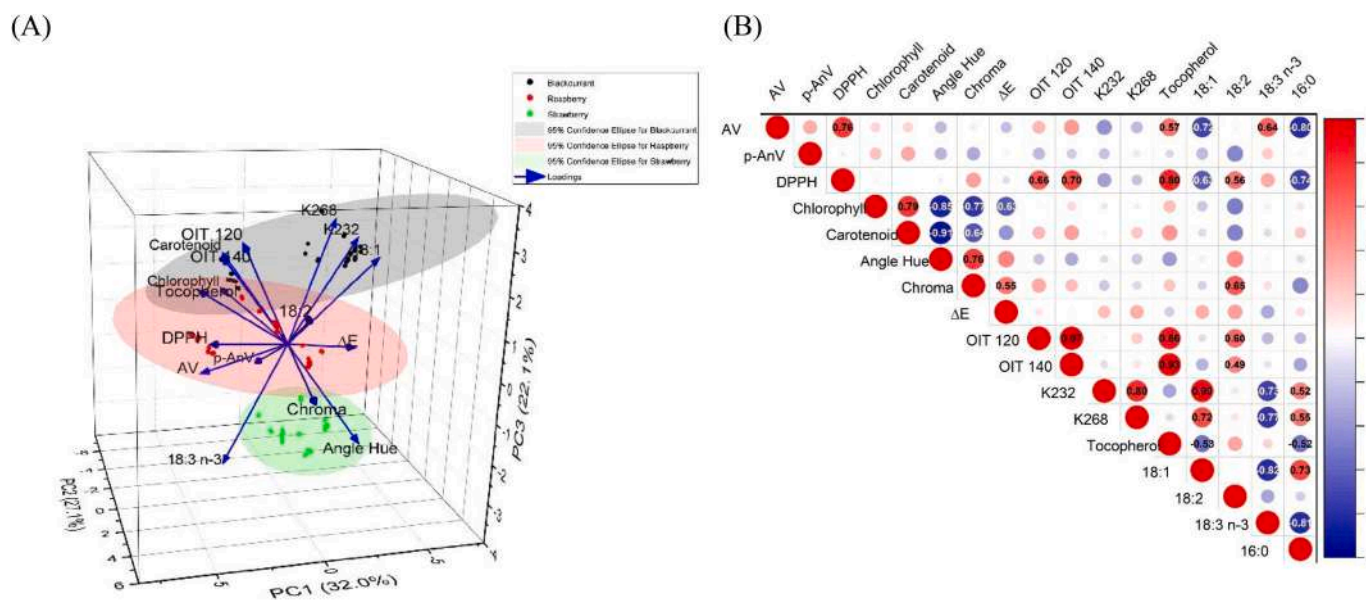


Fig. 3. (A) Biplot 3D from principal component analysis and (B) Pearson's correlation of all tested parameters of berry seed oils throughout storage.

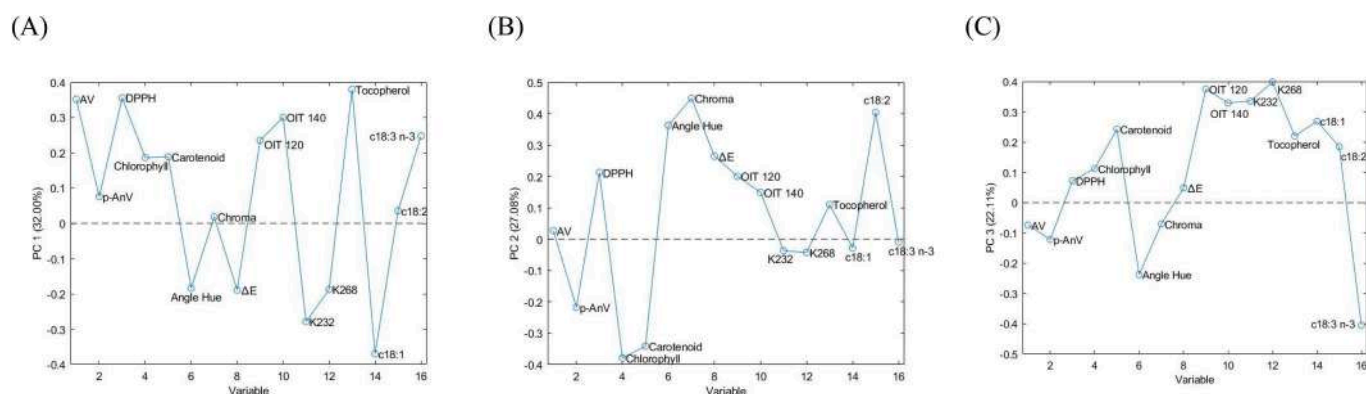


Fig. 4. Loadings plot for PC1 (A), PC2 (B), and PC3 (C).

n-3).

3.2.2. Correlations among quality parameters during storage

Pearson's correlation coefficients were determined for all parameters measured during the storage of BSO (Fig. 3B). Strong positive correlations were observed in thermo-oxidative stability (OIT 120, 140 °C) with DPPH radical scavenging activity ($r = 0.656$, OIT 120; $r = 0.703$, OIT 140) and tocopherols content ($r = 0.858$, OIT 120; $r = 0.927$, OIT 140). The correlations imply that berry seed oils with high DPPH radical scavenging activity and tocopherol content would exhibit a high value of OIT, as presented in Figs. 1E, K, L and 2B. Considering the role of tocopherols in maintaining the overall quality parameters in store-like conditions, these correlations imply the importance of determining the shelf life of berry seed oils based on tocopherol content. In this case, the shelf life of strawberry oil would be shorter compared to that of blackcurrant and raspberry oil.

During storage, the colour intensity in berry oils decreased as the result of pigment degradation, which was confirmed by the strong negative correlations between colour parameters (angle hue, chroma, ΔE) with bioactive compounds content (carotenoid, chlorophyll). It is also worth mentioning the negative correlations between polyunsaturated fatty acids (c18:3 n-3) with the formation of oxidation products such as K₂₃₂ ($r = -0.727$) and K₂₆₈ ($r = -0.774$) during storage. In contrast, positive correlations between monounsaturated fatty

acids (c18:1) with K₂₃₂ ($r = 0.899$) and K₂₆₈ ($r = 0.718$) were observed. Even though FA content remains relatively stable for one year, correlation analysis hints at the possible FA composition trend in prolonged storage conditions. In the case, when PUFA content decreased, for instance due to deterioration, oxidation products were formed, which can be observed by the increases in K₂₃₂ and K₂₆₈. Similar correlations were reported by Zhang et al. [46] with reference to α -linoleic degradation and the increase in K₂₃₂ and K₂₆₈ during thermal-induced oxidation.

3.3. Factors influencing thermo-oxidative stability during storage

As Pearson's correlations were limited to showing the bivariate linear relationship between two variables, overall factors influencing the thermo-oxidative stability of berry oils during storage were investigated using multiple regression (MLR) analysis. Forward stepwise regression was performed to identify the parameters that best explain the thermo-oxidative stability of BSO during storage (Table 1). This hierarchical regression eliminated variables that do not meet the criteria ($p \leq 0.05$), and the models only include potential predictors with statistically significant p -values and non-significant predictors with close values to the criteria. In this case, the models in Table 1 have eliminated chroma and c16:0 indicating the absence of its influence in thermo-oxidative stability. MLR analysis was performed to formulate a general linear

Table 1

Multiple regression analysis (MLR) parameters for independent physicochemical and compositional variables against OIT values of berry seed oils measured at 120 and 140 °C.

OIT (°C)	R ²	Intercept		AV	p-AnV	DPPH	Chl	Cr	h _{ab}	ΔE	K ₂₃₂	K ₂₆₈	Toc	18:1	18:2	18:3 n-3
120	0.973	−215.37×	b	0.53	0.95×	1.05×	−0.68×	4.17×	1.31×	0.05	–	−106.25×	0.33×	3.48×	0.98×	–
			b*	0.09	0.09	0.30	−0.21	0.61	0.26	0.04	–	−0.39	0.82	0.88	0.23	–
140	0.957	−6.07×	b	0.41×	0.24×	–	−0.08×	–	–	–	−1.73×	−16.10×	0.12×	0.68×	–	−0.19×
			b*	0.26	0.08	–	−0.09	–	–	–	−0.24	−0.22	1.10	0.63	–	−0.29

AV: acid value, AnV: p-anisidine value, DPPH: DPPH radical scavenging activity, Chl: chlorophyll, Cr: total carotenoid, h_{ab}: angle hue, Toc: total tocopherols, b: raw regression coefficient, b*: standardized regression coefficient. Statistically significant values were indicated by (×) with $p \leq 0.05$.

equation, which will fit the all variables against oxidation induction time (OIT). This will provide the possibility to predict the thermo-oxidative stability of the oils.

The regression model to predict OIT at 120 °C was statistically significant with $F = 262.42$ ($p\text{-value} \leq 0.05$), which consisted of statistically significant variables i.e. p-AnV, DPPH radical scavenging activity, carotenoid, hue, tocopherol, c18:1, c18:2, chlorophyll, and K₂₆₈. For OIT measured at 140 °C, the model was significant with $F = 225.86$ ($p\text{-value} \leq 0.05$). It contains a smaller number of significant variables, such as AV, p-AnV, tocopherol, c18:1, chlorophyll, oxidation products (K₂₃₂, K₂₆₈) and c18:3 n-3. In addition to the raw regression coefficients (b) of the MLR equation to predict thermo-oxidative stability at various temperatures (120, 140 °C), Table 1 also contains standardized coefficients (b*), which indicate the importance of each independent variable. For OIT at 120 °C, the highest b* values among the independent variables were shown in fatty acid c18:1 (0.88), tocopherol content (0.82), and carotenoid (0.61), respectively, whereas the most important independent variables affecting OIT at 140 °C were tocopherol content (1.10) and fatty acid c18:1 (0.63).

In short, the thermo-oxidative stability of berry seed oils during storage was affected by different parameters, depending on the isothermal temperatures. However, the two models still share some similarities regarding negative (chlorophyll, K₂₆₈ value) and positive (tocopherol, carotenoid, c18:1) relationships. The thermo-oxidative stability of berry seed oils can be maintained during prolonged storage time as long as the tocopherol content is sufficient. Prior to the optional refining process, the berry seed oil producer should be aware of the targeted tocopherol amount in the finished product.

4. Conclusions

As a valorised by-product, the initial quality state of BSO depends heavily on the raw material quality prior to oil extraction. BSO exhibited excellent stability during one year of storage in store-like conditions, despite a minor reduction in the antioxidant activity (DPPH radical scavenging activity, tocopherols and carotenoids content), as well as the increase in oxidation products (p-AnV, K₂₃₂, K₂₆₈ values) and free fatty acids (acid value). The oil's colour became visibly distinctive after 6 months, as observed in the reduction in chlorophyll and carotenoid content, and the increase in colour parameters (hue, chroma, ΔE). Additionally, no major oxidative deterioration occurred in all the oils, as confirmed by the unchanged thermo-oxidative stability, and stable fatty acid composition throughout the year, which may be due to the significant amount of remaining tocopherol. The MLR analysis revealed that the most important parameters influencing the OIT values were oleic fatty acid c18:1, tocopherol and carotenoid content. Summing up, this study shows that many different factors played an important role in maintaining a constant level of the oxidative stability of berry seed oils over a period of one year.

Ethical statement

This study did not include any human subjects and animal experiments.

CRediT authorship contribution statement

Yolanda Victoria Rajagukguk: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mahbuba Islam:** Investigation. **Anna Grygier:** Investigation. **Aleksander Siger:** Investigation. **Magdalena Rudzińska:** Investigation. **Jolanta Tomaszewska-Gras:** Writing – review & editing, Validation, Resources, Methodology, Funding acquisition, Formal analysis, Conceptualization.

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

Data will be made available on request.

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Rapid determination of the storage time of cold-pressed berry seed oils using flash gas chromatography E-Nose coupled with chemometrics

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ABSTRACT

Since oils containing a high content of polyunsaturated fatty acids are susceptible to oxidation, it is necessary to monitor the degree of deterioration during storage, e.g. by measuring the volatile compounds. This study aimed to assess volatile profiles of berry seed oils in terms of the authenticity and the deterioration assessment using flash gas chromatography (FGC E-Nose) combined with chemometrics. Berry seed oils (raspberry, blackcurrant, strawberry, chokeberry), obtained from three different suppliers and stored for a one year in brown bottles at room temperature, were analysed after 0, 3, 6, 9 and 12 months of storage. Principal component analysis enabled separation of oil samples by different berry types, suppliers and storage times. To predict the storage time, partial least square (PLS) models were built for each type of berry oil. Determination coefficients (R^2) in cross-validation ranged from 0.842 (RMSECV = 1.69 months) to 0.969 (RMSECV = 0.75 months). Selecting the specific regions of chromatograms improved the residual prediction deviation (RPD) to values between 2.8 and 5.7, which indicated the suitability of the PLS models to predict the storage time in the quality control of berry oils.

1. Introduction

World production of berries was increased during the past 10 years. The growth in production was observed in the increment of gross production value that ranged up to 25% and 125%, respectively, for strawberry and raspberry commodities (FAO, 2021). Berries were often consumed directly or processed into other convenient products (juice, dried berries, confiture, and marmalade). Agro-industrial processing resulted to the accumulation of by-products such as pomace and other residues (seeds, leaves, stems) that account up to 35% of the raw mass (Majerska et al., 2019). Furthermore, up to 70 % of berry by-products consist of seeds containing a notable amount of oil (Mazurek et al., 2022). Oils from berry seeds are characterized by a high percentage of polyunsaturated fatty acid (PUFA) and antioxidant components, which are linked to health-promoting properties, such as reducing the risk of cardiovascular disease (Martysiak-Zurowska and Orzolek, 2023).

Berry seed oils are sold as food supplements, nutraceutical products, and cosmetics. Despite the growing demand, only a limited number of studies exist on berry seed oils' authenticity (Przykaza et al., 2021; Rajagukguk et al., 2023). The quality of berry seed oil sold on the market remains unprotected due to the non-existing regulation of oils from

by-products. The high profitability of berry seed oil production might attract fraudulent enterprises to tamper with the oils. In fact, fat and oil products remain the third-most reported commodities in terms of the suspicion of fraud in the latest report from the EU-Food Fraud Network, with "faulty storage conditions" and "unsuitable organoleptic characteristics" included in the list of non-compliance categories (European Commission, 2021). From the industrial point of view, developing a reliable method for evaluating storage time is critical to ensure oil quality and to protect the business-to business marketplace against fraud cases.

During storage, oils with higher PUFA are more susceptible to oxidation. The deterioration in quality is mainly indicated by the presence of rancid odours from lipid oxidation products such as aldehydes, ketones, and esters, as well as furan derivatives (Gaca et al., 2021). Quality and authenticity assessment of oils was reported in recent studies using various methods of gas chromatography techniques (Mota et al., 2021). Due to its simplicity, efficiency, and affordability, the flash gas chromatography (FGC) E-nose application has gained attention as a non-targeted instrument to assess the quality of high-lipid-containing products (Barbieri et al., 2020; Cevoli et al., 2022; Tata et al., 2022). FGC E-Nose is a versatile tool that enables rapid analysis of the samples

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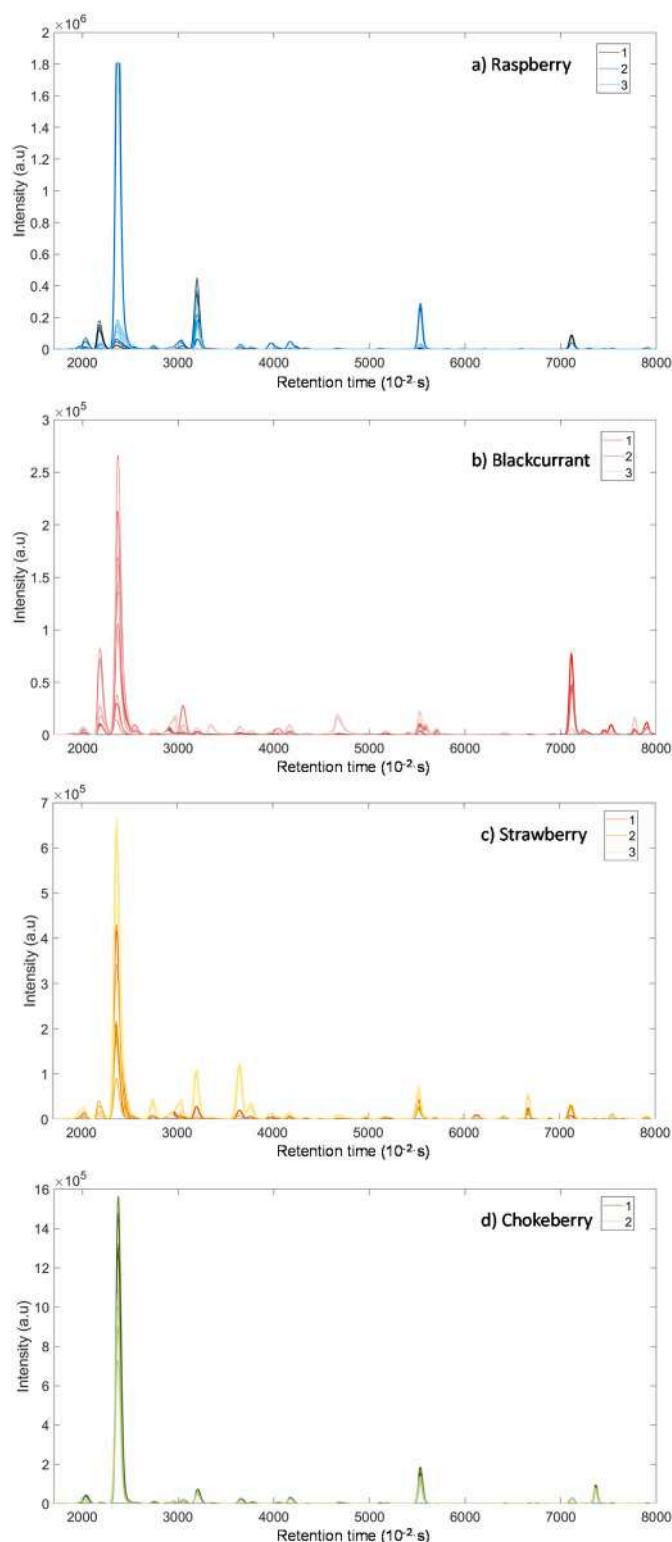


Fig. 1. Chromatogram of a) raspberry, b) blackcurrant, c) strawberry, d) chokeberry seed oils from different suppliers.

compared to conventional GC. The synergy between FGC and E-Nose instruments leads to better data acquisition, i.e. enabling researchers to obtain more specific and complex volatile signals from flavour attributes compared to sensor-based E-nose (Tian et al., 2023). Furthermore, FGC is easy to operate and suitable for profiling volatile compounds, which can be evaluated with chemometric analysis (Damiani et al., 2020). The implementation of FGC E-Nose is still a hot topic in edible oil studies.

The technique capabilities were proven in determining olive oil authenticity (Palagano et al., 2021), the origin of flavoured rapeseed oil (Zhang et al., 2020), and has even been encouraged for use as a replacement for conventional sensory evaluations (Modesti et al., 2021). Further investigation of the implementation of FGC E-Nose to assess edible oil quality will benefit the oil industry.

The objectives of this study were: a) to predict the storage time of berry seed oils using FGC E-nose combined with multivariate data analysis and, b) to discriminate four types of berry seed oils obtained from different suppliers based on the oils' authentic volatile profiles. The samples used in this study consist of cold-pressed oil from raspberry (RB), strawberry (ST), blackcurrant (BC), and chokeberry (CHB). Chromatogram data will be subjected to multivariate data analysis to predict the storage time (PLS model) and to assemble the samples according to berry type and supplier origin (PCA). This work presented the first application of FGC E-Nose to evaluate berry seed oil quality in a storage study.

2. Material and methods

2.1. Materials

Four types of cold-pressed berry seed oils were analysed: raspberry (*Rubus idaeus*) [RB], strawberry (*Fragaria ananassa*) [ST], blackcurrant (*Ribes nigrum*) [BC], and chokeberry (*Aronia melanocarpa*) [CHB]. For each oil type, three different batches of production (GR1, GR2, and GR3) coming from two supplier (GreenField Sp z.o.o., Warsaw, Poland; Olvita Gołuch Sp. k., Marcinowice, Poland), were investigate, except that for the chokeberry seed oil. In this last case two batches were considered. Freshly produced oils were stored for a one-year observation in brown-glass bottles (100 ml) without any headspace at ± 20 °C. The samples were collected from different bottles every 3 months. In total, 110 samples were collected (two replicas for storage time) and analysed after 0, 3, 6, 9, and 12 months of storage.

2.2. Flash gas chromatography (FGC) analysis

Non-targeted volatile compounds analysis was conducted using a flash gas chromatography technique and the Heracles II E-Nose instrument (Alpha MOS, Toulouse, France). FGC is equipped with two capillary columns (10 m length, 180 μ m diameter) containing different stationary phase polarities. Non-polar (MXT5: 5% diphenyl, 95% methylpolysiloxane) and polar (MXT-1701 14% cyanopropylphenyl/86% dimethyl polysiloxane) columns were parallelly separated into compounds with a distinctive capability. The separated volatile compounds were detected by flame ionisation detector (FID).

Berry seed oils (± 2 g) were weighed inside a 20 mL clear-glass vial and sealed with a magnetic cap. Each sample was analysed in two replications. The vials were placed inside a shaker oven for 20 min (40 °C, 500 rpm). Volatile compounds (5 mL) were collected from the vial's headspace using syringe (70 °C) equipped with a splitless injector (200 °C, injection speed 100 μ L/s, carrier gas flow at 30 mL/min) and a Tenax TA® trap (40 °C, 60 s) as the adsorbent. Afterwards, volatile compounds were desorbed from the trap (240 °C, 93 s), injected (column head pressure was set at 40 kPa), and split into two columns (flow 5 mL/min). The analysis was conducted using the following thermal program: 1) start at 40 °C and hold for 2 s, 2) increase the temperature to 80 °C at 1 K/s, 3) increase the temperature to 250 °C at 3 K/s. The carrier gas used was hydrogen (40 kPa–64 kPa, with increasing rate of 0.2 kPa/s). The compounds were detected by a flame ionisation detector (FID) at 260 °C. Alphasoftware version 14.5 was used to record the signals and control the whole process of data acquisition (signal digitalized every 0.01 s).

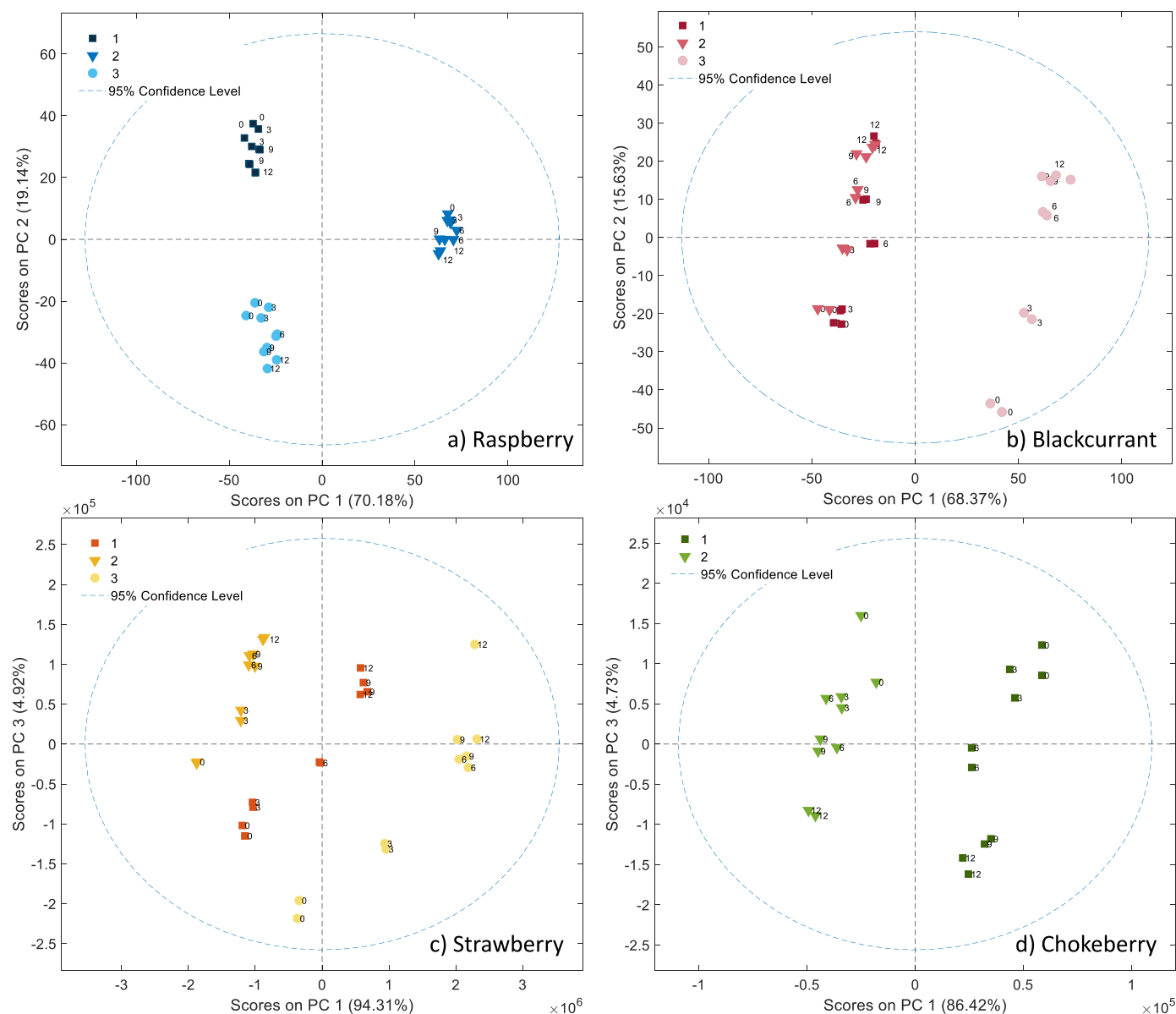


Fig. 2. PCA score plots for a) raspberry, b) blackcurrant, c) strawberry, d) chokeberry seed oils during 0, 3, 6, 9, 12 months of storage.

2.3. Data analysis

By using a non-targeted approach, full chromatograms or some sections of them were used to predict the storage time and to discriminate the sample according to berry types and batch origin. The choice of using full chromatograms has some advantages related to possible errors generated during the integration in peak-area calculation; furthermore, when no pre-selection is done, the risk of discarding useful information is avoided (Melucci et al., 2016). Before the chemometric analysis, the chromatograms belonging to the same berry types were aligned by the COW (Correlation Optimized Warping) algorithm (Tomasí et al., 2004) and pretreated using two methods: Pareto-scaling or simply centering (mean-centering). Pareto-scaling was selected instead of standard auto-scaling because it gives equal importance to all variables, but to a lesser extent than standard autoscaling, which may induce a loss of significant information, since it increases the weights of minor noisy variables (Aliakbarzadeh et al., 2016). Furthermore, by using this pre-processing, the shapes of the chromatograms were not modified (van den Berg et al., 2006). Particularly, in Pareto-scaling the square root of the standard deviation is used as scaling factor:

$$\tilde{x}_{ij} = \frac{x_{ij} - \bar{x}_i}{\sqrt{SD_i}} \quad (1)$$

where x_{ij} and $\tilde{x}_{ij}$ represent the raw and scaled data, respectively. The mean ($\bar{x}_i$) and standard deviation (SD_i) are calculated as:

$$\bar{x}_i = \frac{1}{J} \sum_{j=1}^J x_{ij} \quad (2)$$

$$SD_i = \sqrt{\frac{\sum_{j=1}^J (x_{ij} - \bar{x}_i)^2}{J - 1}} \quad (3)$$

Explorative PCA models were built to visualize samples according to berry type and batch origin, while PLS models were developed to estimate the storage time within the same berry type, considering all suppliers together. In view of the small number of samples, the PLS model validation were performed by Venetian blinds cross-validation (10 segments). To avoid the model over-fitting, the optimal number of latent variables (LV) were chosen by detecting the global minimum of root

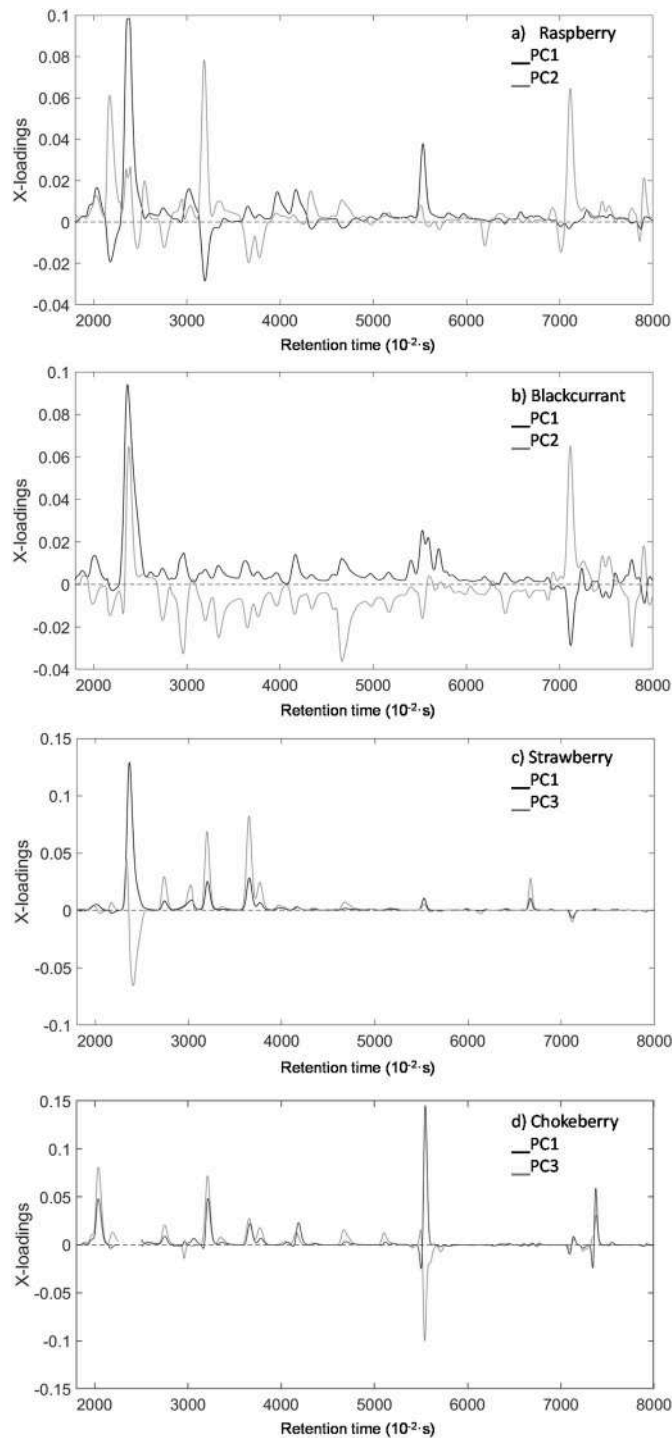


Fig. 3. X-loadings score plots obtained by the PCA of the a) raspberry, b) blackcurrant, c) strawberry, d) chokeberry data.

mean square error in cross validation (RMSECV).

Furthermore, the model robustness and significance was verified through a permutation test. This procedure allows overfitting to be identified and provides the probability that the given model is significantly different from one built under the same conditions but using random data.

It is often the case that most of the variables involved in the PLS model development could be of slight relevance (e.g. redundant or unnecessary chromatogram regions) to the investigated problem, as they represent variation not related to the response to be modelled.

Table 1

Results of the PLS models developed using a full chromatogram (1800–8000).

Berry	Pre-processing	Calibration		Cross-validation		LV	
		R ²	RMSEC (months)	R ²	RMSECV (months)	RPD	
RB	PA + MC	0.969	0.75	0.842	1.69	2.5	8
BC	PA + MC	0.984	0.54	0.846	1.63	2.5	11
ST	MC	0.985	0.5	0.932	1.14	3.8	11
CHB	PA + MC	0.995	0.26	0.963	0.82	5.2	8

RB: raspberry; BC: blackcurrant; ST: strawberry; CHB: chokeberry; PA: Pareto Scaling; MC: mean-centering; R²: determination coefficient; RMSE: Root Mean Square Error; C: calibration; CV: cross-validation; RPD: residual prediction deviation; LV: latent variables.

Therefore, their number can be drastically reduced without loss of information, or even increasing the model power. Variable selection can improve the estimation accuracy by effectively identifying the subset of important predictors and can enhance the model's interpretability with parsimonious representation (Farrés et al., 2015). There are several methods to select variables, and one of those most used in combination with PLS regression is the Variable importance in projection (VIP) selection method. VIP scores summarize the influence of individual X-variables on the PLS model. They are calculated as the weighted sum of squares of the PLS weights, which take into account the amount of explained Y-variance in each extracted latent variable (component).

The VIP score for each j variable is calculated as:

$$VIP_j = \sqrt{\frac{\sum_{f=1}^F w_{jf}^2 \bullet SSY_f \bullet J}{SSY_{tot} \bullet F}} \quad (4)$$

where w_{jf} is the weight value for j variable and f component, SSY_f is the sum of squares of explained variance for the fth component and J number of X variables. SSY_{tot} is the total sum of squares of explained variance of the dependent variable, and F is the total number of components.

X variables characterized by VIP scores higher than 1 are considered important in a given model; this criterion is conventionally used to select the variable. Accordingly, new PLS models involving only the X variables with VIP scores greater than one were developed.

The results were evaluated in terms of determination coefficient (R²), root mean square error in (RMSE), and residual prediction deviation (RPD) in calibration (C) and validation (CV)

$$R^2 = \frac{\sum_{i=1}^N (\hat{y}_i - \bar{y})^2}{\sum_{i=1}^N (y_i - \bar{y})^2} \quad (5)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^N (\hat{y}_i - \bar{y})^2}{N}} \quad (6)$$

$$RPD = \frac{SD}{RMSE} \quad (7)$$

where y_i is the actual storage time (days), $\hat{y}_i$ is the predicted storage time (days), $\bar{y}$ is the mean of the actual values, N is the number of samples, and SD is the standard deviation of reference values. All data analyses were conducted using PLS Toolbox for Matlab2018a®.

3. Results and discussion

All chromatograms, grouped based on the berry type, are shown in Fig. 1. The main peaks are concentrated in the initial part of the chromatogram (retention time between 18 and 80 s). As could be expected, the volatile profiles of the four types of oils (RB, BC, ST and CHB) are quite different, in terms of shape and peak numbers. Clear differences between supplier origin can be observed for all berry types, especially in

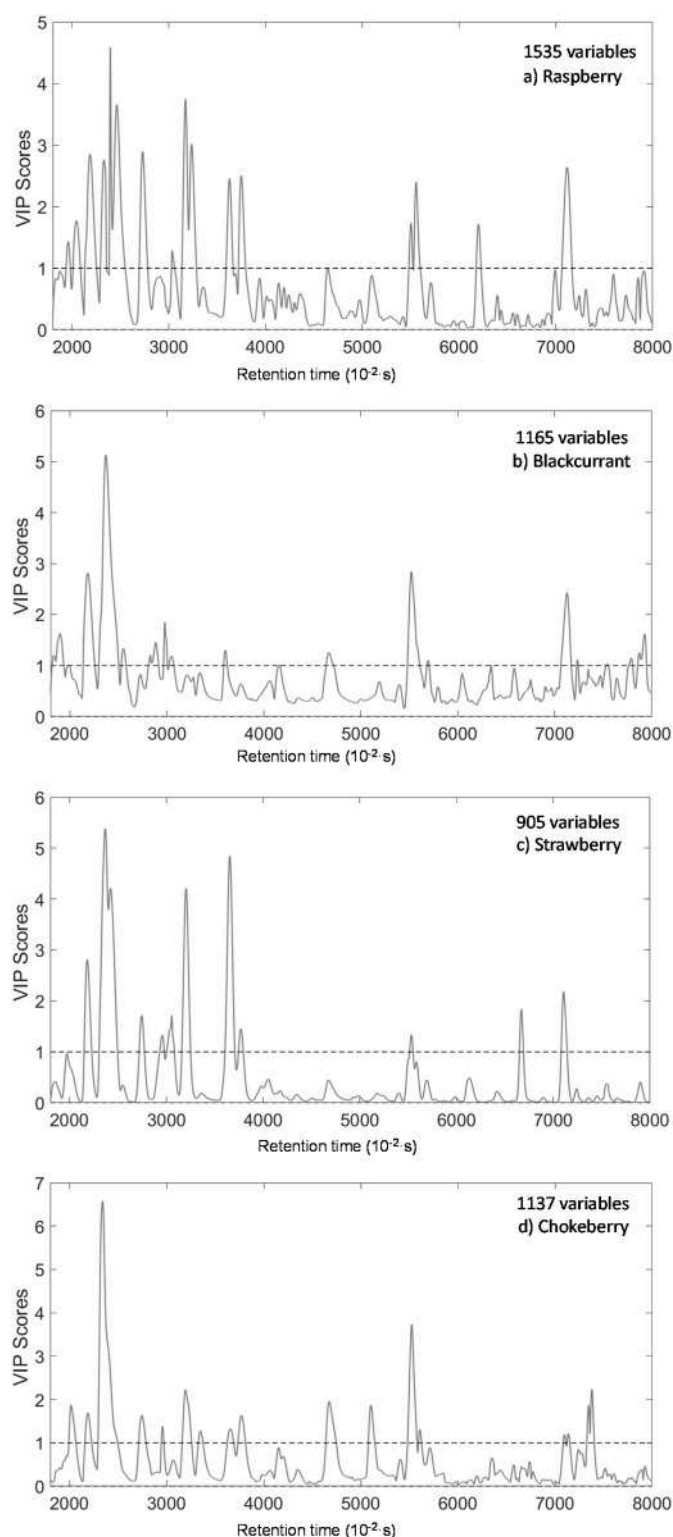


Fig. 4. VIP scores from the selected regions in chromatogram for a) raspberry, b) blackcurrant, c) strawberry, d) chokeberry seed oils.

terms of intensities, confirming the discriminating power of the volatile profile with respect to the supplier. This draws attention to the fact that sample oils obtained from two different batches of production from the same supplier (group 1 and 2) are characterized by different volatile profiles, even immediately after production.

Explorative PCAs were conducted to evaluate a possible correlation between chromatograms according to berry type and supplier origin.

Score plots obtained for the chromatogram range from 1800 to 8000 points (retention time from 18 to 80 s) are shown in Fig. 2. Better results were achieved for pre-processing RB and BC data by Pareto-scaling, and for ST and CHB data by mean-centering. For all berry types, a good separation was observed for the supplier origin PC1 vs PC2 (RB and BC) or PC1 vs PC3 (ST and CHB). For the BC samples, it is difficult to discriminate between group 1 and 2, while group 3 shows high differences with respect to the other two. This is probably due to the fact that samples belonging to group 3 are produced by a different company.

Within the same supplier, it is also possible to observe quite good distribution of the samples as a function of storage time, from 0 to 12 months, especially along the PC2 (RB and CB) or PC3 (ST and CHB).

The contribution of X-variable to each of the PCs can be evaluated by the X-loading. High loading values (positive or negative) indicate that a variable has a strong effect on that principal component. Positive loadings indicate a positive correlation between variable and principal component: an increase in one results in an increase in the other. Negative loadings indicate a negative correlation. Therefore, by evaluating the X-loadings (Fig. 3), it was possible to observe the chromatographic zones characterized by the highest contribution to PC1 vs PC2 (RB and CB) or PC1 vs PC3 (ST and CHB). The presence of greater noise in the loading scores of the RB and CB samples compared to the ST and CHB, is due to the data pre-treatment (Pareto-scaling vs mean centring).

A brief attempt to identify the FGC E-Nose peaks from olive oils of different geographical origin was reported in the study conducted by Melucci et al. (2016). The authors compared the FGC E-Nose peaks with MS spectra in SPME/GC-MS analysis and successfully identified a positive correlation between retention times and particular molecules. According to Melucci et al. (2016), a rough identification of the responsible molecules for some prominent peaks from FGC E-Nose in Fig. 1 can be established. The identified peaks were listed as follows: a) ethyl acetate at 2100, b) ethanol 2200, c) 1-penten-3-ol at 2400, d) hexanal at 3200, e) hexanol at 3600, and f) 2-hexenal at 4100.

Considering the discrimination according to the storage time (observed along PC2 or PC3), the highest contribute is mainly due to the peaks at around 2200, 3200 and 7100; 2400 and 7100; 2400, 3200 and 3600; as well as 2100, 2400, 3200 and 5600, for RB, BC, ST and CHB, respectively. In this regards, several prominent volatile compounds that responsible for storage time differentiation as detected by FGC E-Nose are 1-penten-3-ol, hexanal, and 2-hexenal. Hexanal and 2-hexenal were generated from the oxidation of linoleic and linolenic acid respectively (Xu et al., 2018), which responsible for up to 75% of the fatty acid composition in berry seed oils (Mildner-Szkudlarz et al., 2019). While the increment of 1-penten-3-ol over time indicates the decomposition of hydroperoxides from omega-3 fatty acid in berry seed oils (Liang et al., 2020). Beside quality deterioration and off-flavours, the detected volatiles were also responsible for the key-aromas that set apart one type of berry seed oil among the other oils. For example 1-hexanol (fruit, banana, soft, tomato, cut grass), 2-hexenal (green, apple-like, bitter almond like), and hexanal (apple, cut grass, green) (Marx et al., 2021; Teixeira et al., 2021). Regardless the detected peaks in the presented chromatogram, it should be noted that FGC E-Nose is a completely non-targeted method. Hence, a non-targeted approaches was used to elaborate the data, consequently the precise identification of the volatile compounds related to the peaks is outside the scope of the work. However, this study does not exclude the fact that further studies could be done on the analytical determination of these volatile compounds from a purely chemical point of view.

PLS models were developed to estimate the storage time (months) within the same berry type, regardless of the supplier origin, by using full chromatograms (from 1800 to 8000 ponits). Model results, in terms of determination coefficient (R^2), RMSE, RPD and (LV) in calibration and cross-validation (10 segments) are shown in Table 1. Good results were obtained for all berry oils with R^2 in cross-validation ranging from 0.842 (RMSECV = 1.69 months, RB) to 0.963 (RMSECV = 0.82 months, CHB), while RPD values are equal to or higher than 2.5 (up to 5.2 for

Table 2
Results of the new PLS model developed using a selected region in the chromatogram by the VIP method.

Berry	Pre-processing	Numbers of variable	Calibration		Cross-validation			LV
			R ²	RMSEC (months)	R ²	RMSECV (months)	RPD	
RB	PA + MC	1535	0.980	0.60	0.973	1.01	4.0	5
BC	PA + MC	1165	0.970	0.73	0.932	1.14	3.8	7
ST	MC	905	0.973	0.68	0.895	1.42	3.1	4
CHB	PA + MC	1137	0.989	0.44	0.969	0.75	5.7	5

RB: raspberry; BC: blackcurrant; ST: strawberry; CHB: chokeberry; PA: Pareto Scaling, MC: mean-centering; R²: determination coefficient; RMSE: Root Mean Square Error; C: calibration; CV: cross-validation; RPD: residual prediction deviation; LV: latent variables.

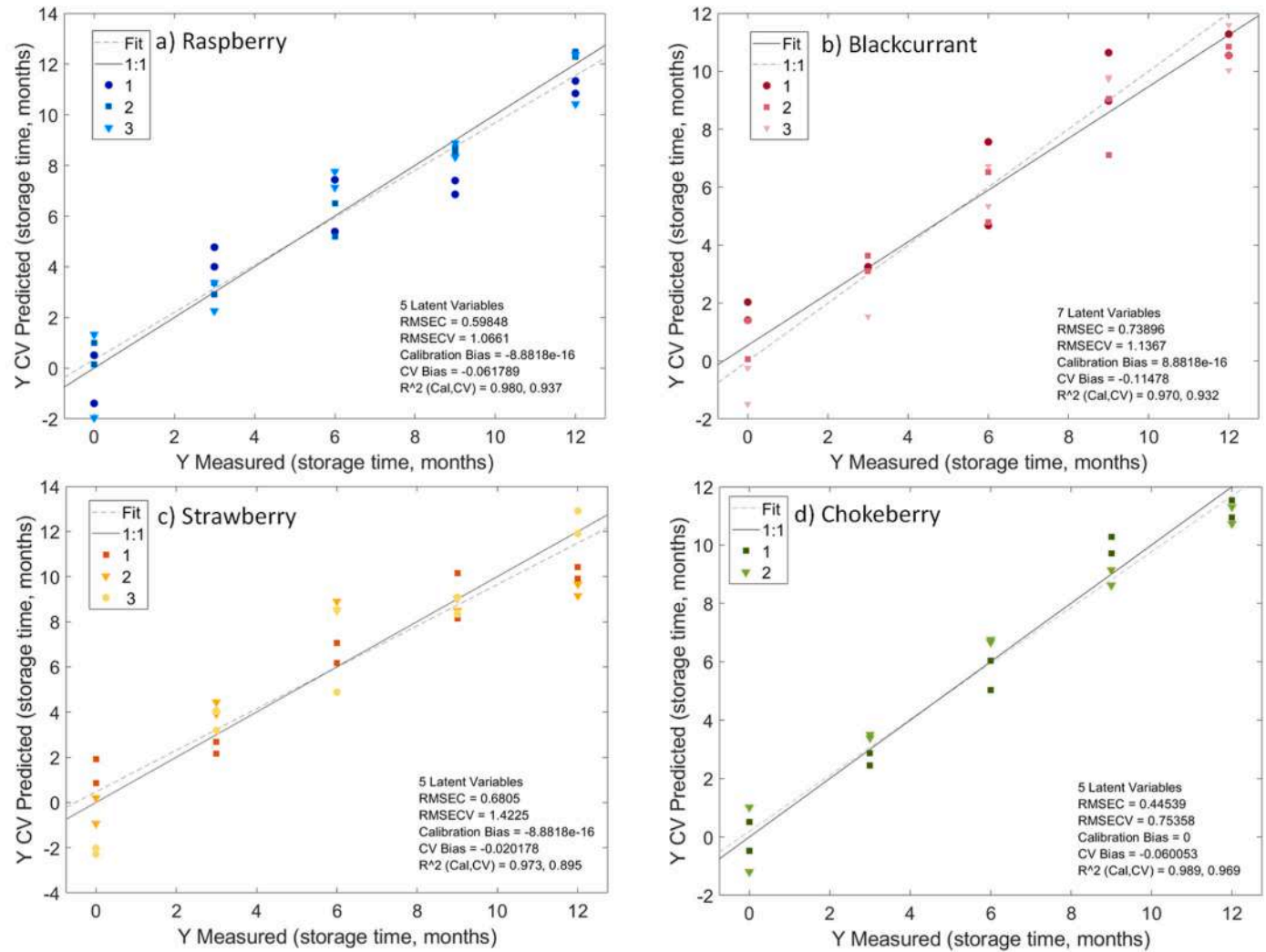


Fig. 5. PLS results in terms of measured versus predicted storage (time months) values for a) raspberry, b) blackcurrant, c) strawberry, d) chokeberry data.

Table 3
The probabilities of the calibration and cross-validation models.

	Berry	Calibration			Cross-validation			LV
		Wilcoxon	Sign Test	Rand t-test	Wilcoxon	Sign Test	Rand t-test	
Full chromatograms	RB	0.000	0.008	0.005	0.000	0.003	0.005	8
	BC	0.000	0.011	0.005	0.000	0.016	0.005	11
	ST	0.001	0.006	0.005	0.000	0.002	0.005	11
	CHB	0.000	0.003	0.005	0.000	0.002	0.005	8
After VIP variable selection	RB	0.000	0.007	0.005	0.000	0.001	0.005	5
	BC	0.000	0.007	0.005	0.000	0.004	0.005	7
	ST	0.001	0.008	0.005	0.000	0.003	0.005	4
	CHB	0.000	0.002	0.005	0.000	0.002	0.005	5

Note: RB: raspberry; CB: chokeberry; ST: strawberry; BC: blackcurrant; LV: latent variables.

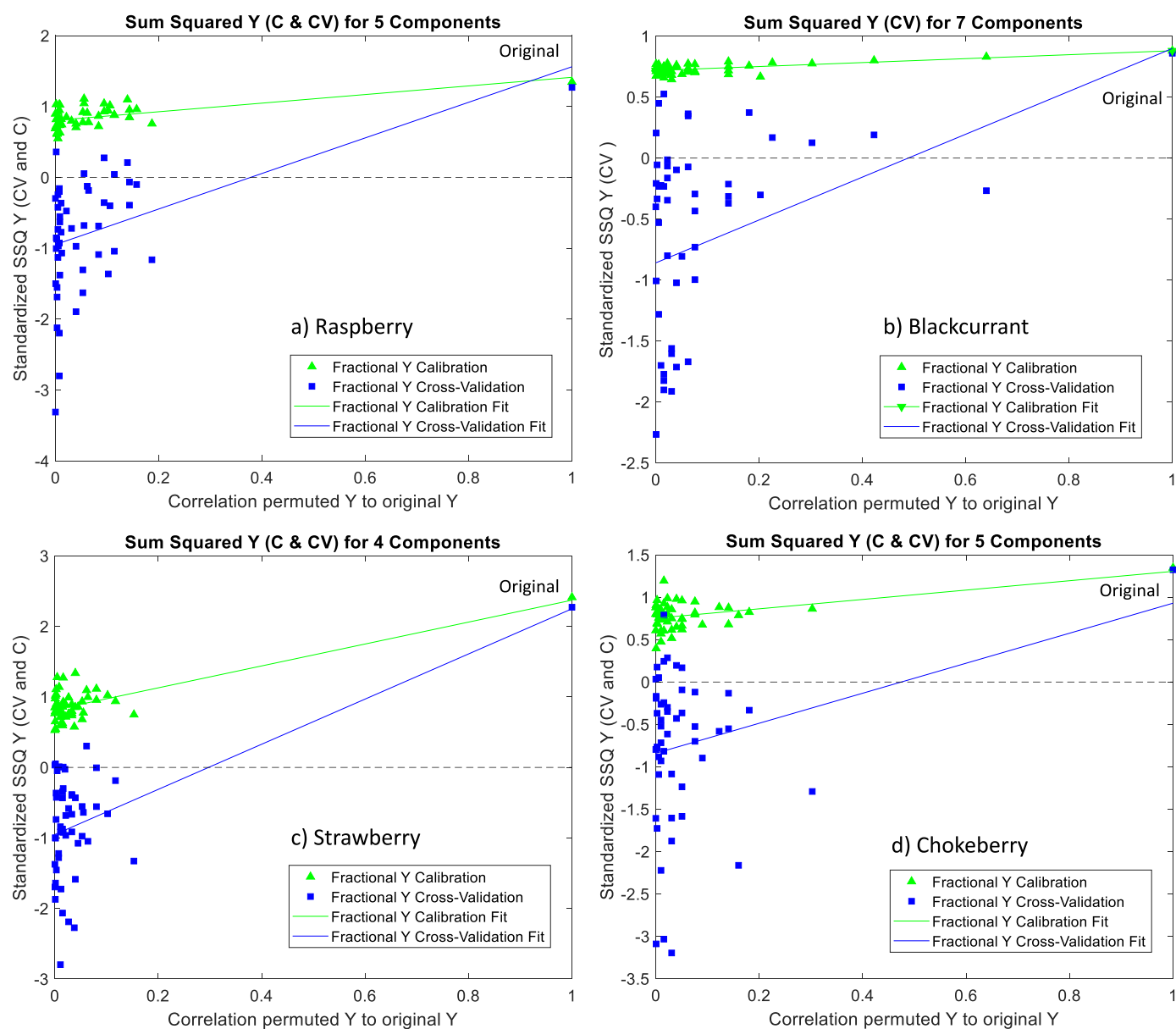


Fig. 6. Fractional y-variance from calibration and cross-validation versus correlation of the permuted y-block to original y-block in a) raspberry, b) blackcurrant, c) strawberry, d) chokeberry seed oils.

CHB samples).

With the aim of reducing the original data set, removing redundant or unnecessary chromatogram regions, and thus increasing the PLS model predictive power, variables characterized by $VIP > 1$ were selected and new PLS models were developed. Chromatograms for selected regions are shown in Fig. 4. The number of variables was reduced by more than 75% (1535 variables) and up of 85% (905 variables) with respect to the original data set (6200 variables). The number of LV is also considerably reduced (from 9.5 ± 1.5 to 5.2 ± 1.1) making the models more stable. The results of the new PLS models are shown in Table 2. For all the samples, except ST, results in cross-validation were improved (mean RMSECV reduction of $26.3 \pm 13.2\%$) compared to those obtained for full chromatograms. R^2 ranges from 0.895 (RMSECV = 1.42 months, ST) to 0.969 (RMSECV = 0.75 months, CHB), while RPD values vary between 3.1 and 5.7. The results in terms of measured versus predicted storage time (months) of the cross validated models are reported in Fig. 5. Furthermore, determination coefficients (R^2) RMSE and BIAS in calibration and cross-validation are shown.

Although there is no statistical basis as to how the threshold equal to

two was determined, usually models characterized by RPD values higher than two are considered as excellent. Furthermore, for the quality control field, the following RPD categories have been identified: i) 2.4–3.0 rough screening quality; ii) 3.1–4.9 screening quality; iii) 5.0–6.4 quality control; iv) 6.5–8.0 process control; v) > 8.1 any application (Williams and Norris, 2001). Considering the RPD values achieved in this study, the PLS models could be suitable for predicting the storage time in quality control.

Due to the restricted number of the samples, it was important to evaluate the robustness and significance of the model as a function of the latent variable, and also to avoid overfitting. In particular, the model robustness and significance was verified through a permutation test that shows the probability of the original model (unpermuted) being significantly different from the one built under the same conditions but using random data (permuted model). The probability that the unpermuted model is not significantly different from the one created from randomly shuffling the y-block was evaluated by using three different tests: Pairwise Wilcoxon signed rank test (Wilcoxon), Pairwise signed rank test (Sign Test) and Randomization t -test (Rand t -test). Table 3 shows the

probabilities in calibration and cross-validation, with values less than 0.05 indicating that the model is significant at the 95% confidence level for the specific number of latent variables. It is possible to confirm that all the developed models are significant with values higher than 98%, both in calibration and validation.

Furthermore, fractional y-variance captured for calibration and cross-validation versus the correlation of the permuted y-block to the original y-block was evaluated as an index of model robustness (Fig. 6, PLS models developed after variable selection). For each permuted y-block, RMSEC and RMSECV were used to calculate fractional sum squared Y captured (SSQ Y) for the calibration (C) and cross-validation (CV). In general, the cross-validated and calibration SSQ Y values should be relatively close to each other but should be less than the results for the unpermuted y-block (right side of the plot), independent of their correlation with the real class values. Consequently, all the models can be considered significant, though the PLS model related to BC shows less robust results in terms of dispersion of SSQ Y values (both C and CV) and the distance between unpermuted and permuted SSQ Y. This agrees with the probability's values reported in Table 3, especially for the Sign Test.

4. Conclusions

The changes in the volatile profile of berry seed oils during one year of storage were correctly evaluated by FGC E-nose combined with chemometric techniques. A clear separation between samples obtained from different suppliers was demonstrated in PCA. Additionally, samples from 0 to 12 months were well distributed, according to the function of storage time. PLS models were built to predict the storage times for each berry type, and the predictive power was improved after pre-processing and selecting the specific region in a chromatogram. The PLS models built in this study possessed excellent predictive power, as characterised by the RPD values that were greater than 2. It indicates the suitability of the PLS models to predict the storage time during quality control. As a rapid and non-targeted instrument, FGC E-nose proved its robust application to analyse the quality differences of berry seed oils during storage. Data processing with chemometrics was able to extract a wider scope of information from the resulting chromatogram, such as visualisation of oil characteristics according to the berry type, supplier origin, and storage prediction. Even though this study using FGC E-nose is limited to qualitative determination, such an approach is valued by the oil industry, which requires analysis of a great number of oils daily, with respect to their quality and authenticity. FGC E-nose is a versatile tool compared to conventional GC, only 100 s of acquisition time are required and no need for solvents.

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CRediT authorship contribution statement

Yolanda Victoria Rajagukguk: Conceptualization, Methodology, Investigation, Writing – original draft, Writing – original draft. **Chiara Cevoli:** Conceptualization, Data curation, Formal analysis, Methodology, Validation, Writing – original draft, Writing – original draft. **Ilaria Grigoletto:** Methodology. **Jolanta Tomaszewska-Gras:** Conceptualization, Funding acquisition, Methodology, 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

Data will be made available on request.

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Advanced authenticity screening of commercial berry seed oils using full FTIR spectra and DSC curves coupled with chemometrics

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ABSTRACT

The feasibility of Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared (FTIR) spectroscopy coupled with Partial Least Square Discriminant Analysis (PLS-DA) for the authenticity screening of blackcurrant-, strawberry-, and raspberry seed oils was evaluated. Authentic DSC and FTIR fingerprints of berry seed oils were reported and used as training data, reflecting their natural variability. In DSC analysis, full curves of melting phase transition were utilised instead of individual DSC parameters (peak temperature, peak height, enthalpy). Each berry seed oil was characterised by distinctive endothermic and exothermic peaks. A FTIR model was built based on the absorbance intensity at three spectral ranges of 3550–3200 cm^{-1} , 3010–2854 cm^{-1} , and 1720–720 cm^{-1} . With supervised learning and personalised data processing, the classification performance of both DSC and FTIR were improved. During external validation using commercial oils, the DSC models demonstrated good predictive performance in classifying blackcurrant and strawberry oils, while FTIR models were favoured for predicting the class of blackcurrant and raspberry oils. This work provides industry practitioners with practical insights into processing the thermal and spectral fingerprint data for the authenticity screening of berry seed oils.

1. Introduction

Analytical methods for food authentication are divided into two main categories -targeted and non-targeted analysis. Targeted analysis allows direct authentication by quantifying the amount of pre-defined analytical target, such as primary or secondary markers (Ballin & Laursen, 2019). Non-targeted analysis qualitatively determines the authenticity of a sample based on fingerprints associated with specific analytical instruments. These analytical methods were selected based on their suitability for solving the authenticity issues being addressed. The Agri-Food Fraud Network (FFN) categorised authenticity issues into several categories i.e. counterfeit, document forgery, grey market (illegal import, smuggling), mislabelling, and adulteration or tampering (European Commission, 2024, p. 28). Targeted analysis is preferred when a single compound is already identified as the fraud indicator, for example, the presence of 20 % methanol in liquor that caused severe poisoning (Visciano & Schirone, 2021). Non-targeted analysis may provide valuable information when screening unspecified fraud sources, such as the presence of various impurities (brown sugar, barley, rye, and corn) in ground and roasted coffee (de Moura Ribeiro et al., 2017). Due to the specific advantage of using non-targeted analysis, this work

focused on the usefulness of Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared (FTIR) spectroscopy for the authenticity screening of commercial berry seed oils.

As a thermoanalytical instrument, DSC measures the difference in heat needed to raise the temperature of sample and reference material. This difference is then plotted as peaks, it may consist of exothermic or endothermic peaks indicating heat-producing and heat-consuming phenomena, respectively. In food research, DSC was often utilised to analyse the thermal behaviour of fats and oil, carbohydrates, and protein (Islam et al., 2022). The past decade has also seen progressive efforts to couple DSC with chemometrics, the aim being to extract more information about thermal behaviour were observed. Some works even combined DSC with other analytical instruments and chemometrics to obtain a whole new perspective in oil matrices, for example, coupling Partial Least Square (PLS) regression with DSC and gas chromatography (GC) data to determine fatty acid composition (Cerretani et al., 2011) or applying PLS to high-pressure liquid chromatography (HPLC) and DSC data to evaluate the influence of triacylglycerol composition on the shape of DSC curves (Maggio et al., 2014). In DSC authentication studies, several multivariate analyses, namely, Linear Discriminant Analysis (LDA), Principal Component Analysis (PCA), Stepwise Multiple

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Linear Regression (SMLR), were applied to DSC parameters (peak enthalpy, onset cooling/melting temperatures). The aim was to discriminate different cultivars of almond (Sanahuja et al., 2011), evaluate lipid degradation in olive oil (Chiavaro et al., 2013), and determine palm oil adulterants in butter (Tomaszewska-Gras, 2016a), respectively. These studies confirmed that both an optimum DSC instrumental set-up (scanning rate, sample mass, particle size, sample vessel, etc) and chemometrics techniques applied were empirical and designed specifically for each food matrices. Despite these advances, the knowledge to utilise DSC for the authentication of other highly valued oils is rather limited.

Fourier Transform Mid-Infrared (FT-MIR or FTIR) spectroscopy measures molecular vibrations by the absorption of infrared within the spectral range of 4000–400 cm^{-1} . The overall spectrum provides information related to total biochemical composition in food matrices with specific fingerprints present between 1500 and 650 cm^{-1} . FTIR combined with chemometrics techniques were widely used in oil authentication studies. Recent advances in oil authentication using FTIR spectroscopy revealed some optimised methods to improve qualitative and quantitative analysis by chemometric models, for example, utilising specific spectral regions associated with samples' traceability to determine geographical and temporal origins of crude palm oil using OPLS-DA (Rozali et al., 2023) and quantify adulterants in camelina oil by applying a BiPLS algorithm into optimised spectral regions (Ye & Meng, 2022). Unlike DSC melting profiles, FTIR spectra of edible oils look extremely similar to each other. Despite this similar appearance, the optimised methods reported in specific oil matrices do not work universally in every edible oils. This is confirmed by a recent study on pattern recognition methods to analyse FTIR spectra from 20 varieties of edible oil ($n = 97$) (Sota-Uba et al., 2021). Using hierarchical clustering analysis, 20 edible oil varieties were divided into four distinct groups. Olive oils were reported to share similar fingerprint patterns with avocado, peanut, almond, and sunflower oils. While other oils such as canola, sesame, and soybean were clustered into other groups with different fingerprint patterns. Hence, specific patterns in edible oil fingerprints justified the necessity of expanding the knowledge to utilise FTIR for the authentication of other underexplored edible oils.

Berry seed oils (BSO) are one of the valorised products from berry processing. After berries were processed into juices, the remaining by-products, such as pomace and seeds, were dried. The seeds were then isolated from pomace and become a raw material for edible oil industries. Due to the abundant amounts of natural antioxidants, BSO are sold in higher prices in the market. For example, blackcurrant seed oil is sold as food supplement and costs around 25 EUR per pack containing 180 soft capsules. As a highly valued oil, BSO authenticity remains unprotected due to the absence of regulation in force. To date, only small number of studies covered the authentication method specified for BSO. However, in recent years, some advances to explore the authentic characteristic of berry seeds and its extracts were reported. For example, Krstić et al. (2023) explored the authenticity assessment of berry seeds using phenolic profiling by HPLC-MS and multivariate analysis, with Mazurek et al. (2017) also attempting to study the authentic characteristics of berry oil extracts obtained from supercritical carbon dioxide extraction by GC-MS. Additionally, in our previous work about the utilisation of non-targeted analysis for rapid authenticity assessment of BSO, we reported the feasibility of Flash GC E-Nose coupled with PLS regression to predict and discriminate oils according to storage times (Rajagukguk et al., 2024).

This work intends to fill the gap concerning studies covering the non-targeted analysis combined with chemometrics to assess berry seed oil authenticity. Therefore, the first aim of this work is to establish the authentic DSC and FTIR fingerprints of berry seed oils and their natural variability. The second aim is to evaluate the feasibility of DSC and FTIR, coupled with chemometrics, for rapid authenticity screening of berry seed oils. Characteristics of authentic blackcurrant (BC), raspberry (RB), and strawberry (ST) seed oils were obtained from freshly pressed

samples and were used as training data. One of the challenges during BSO authentication is having a sufficient sample size to represent the natural variabilities, causing an overfitting in the developed chemometrics model. Addressing these concerns, we include oils extracted from seeds from different suppliers and production batches to enhance the training data, in addition to using commercial BSO for the external validation. This work provides practical insights for industry practitioners about processing thermal and spectral fingerprints data for the authenticity screening of BSO.

2. Material and methods

2.1. Materials

Three types of berry seeds - blackcurrant (*Ribes nigrum*), strawberry (*Fragaria ananassa*), and raspberry (*Rubus idaeus*) - were purchased from local berry seed suppliers, namely, GreenField Sp. z.o.o (Warsaw, Poland) and Olvita Gołuch Sp. k. (Marcinowice, Poland). A total of 68 oil samples (16 blackcurrant, 26 strawberry, and 26 raspberry) were pressed in-house from different seed batches. These oils were used to build the model of the authentic fingerprints. Additionally, 38 commercial berry seed oils (10 blackcurrant, 10 strawberry, and 18 raspberry) were purchased from the market. These commercial oils were intended to be used as the testing data during authenticity screening. All oils were kept in the brown-glass bottle at -80°C prior to the analysis.

2.1.1. DSC melting profile

The DSC melting profiles were determined based on the method described by Tomaszewska-Gras (2016b). The thermal analysis was carried out using PerkinElmer DSC 7 (PerkinElmer, Norwalk) equipped with an Intra cooler II, Pyris software, and nitrogen (99.99 % purity) as the purge gas. The machine was calibrated using indium (m.p. 156.6°C , $\Delta H_f = 28.45 \text{ J/g}$) and n-dodecane (m.p. -9.65°C , $\Delta H_f = 216.73 \text{ J/g}$). A sample (6–7 mg) was placed in an aluminium pan (PerkinElmer, No. 0219-0062, Waltham, MA, USA) and hermetically sealed. An empty sealed aluminium pan was used as a reference during thermal analysis. Afterwards, the machine was programmed with the following procedure: a) heating at 30°C for 5 min, b) cooling with scanning rate 2°C/min from 30°C to -67°C , c) heating at scanning rate 5°C/min from -67°C to 30°C . The whole curve of the melting profile was analysed to build the model of authentic BSO fingerprints. All samples were analysed in duplicate.

2.1.2. DSC oxidative stability

Thermo-oxidative stability was determined based on the method described in ISO 11357-6 (2018), with a slight modification adjusted for BSO (Rajagukguk, Islam, Siger, et al., 2023). Samples (6–7 mg) was weighed into an open aluminium pan (PerkinElmer, No. 0219-0041, Waltham, MA, USA). The reference used during thermo-oxidative stability analysis was an empty open aluminium pan. Samples were kept at isothermal temperatures of 120°C or 140°C under a constant oxygen exposure at 20 mL/min until the end of oxidation time. Thermo-oxidative stability referred to as oxidative induction time (OIT) was determined in duplicate and expressed in minutes.

2.1.3. FTIR spectra

The FTIR spectra of samples were captured using a Spectrum Two FT-IR spectrometer with universal ATR equipped with a diamond crystal (PerkinElmer, Waltham, MA, USA). The spectra were captured starting from 4000 to 500 cm^{-1} wavenumbers with 32 scans per sample or background, scanned at 4 cm^{-1} resolution in reflectance mode. During data acquisition, the sample ($\pm 50 \mu\text{L}$) was placed onto the top of ATR crystal. The whole FTIR spectrum of each sample was analysed to build authentic BSO fingerprints. The data acquisition was conducted in duplicate.

2.1.4. DPPH radical scavenging activity

Determination of DPPH radical scavenging activity was performed following the method described in [Sánchez-Moreno et al. \(1998\)](#). A sample mixture was prepared by mixing 100 μ L of oil with ethyl acetate (2 mL) and a DPPH radical solution (1 mmol/L, 250 μ L). Afterwards, the mixture was thoroughly vortexed and kept away from light for 20 min. The reading was conducted at 515 nm in triplicate. The result was expressed as μ mol of Trolox equivalent (TE) per gram of oil.

2.1.5. Fatty acid composition

Fatty acid composition was determined following the method described by [AOCS \(2005\)](#). Samples (± 0.01 g) were dissolved in 1 mL of hexane (>99 %, Avantor, Gliwice, Poland) and 1 mL of 0.4 M sodium methoxide (Merck, Darmstadt, Germany). The mixtures were mixed thoroughly and left for 15 min. To stop the reaction, 5 mL of distilled water was added to the mixture and kept for another 5 min until the proper layer separation occurred. The upper layer of the mixture (± 1 mL) was transferred to a gas chromatography (GC) vial and taken for analysis. The GC analysis was carried out using Trace 1300 equipped with an FID detector and capillary column SP TM-2650 with a dimension size of 100 m $\times$ 0.25 mm $\times$ 0.2 μ m (Supelco, Bellefonte, PA, USA). The machine was programmed with the following procedure: a) oven heating to 160 $^{\circ}$ C for 1 min, b) heating to 220 $^{\circ}$ C at 6 $^{\circ}$ C/min. The temperature of inlet and detector was set at 240 $^{\circ}$ C. The carrier gas used during the analysis is hydrogen (1.5 mL/min) gas in splitless mode. Data interpretation was performed by comparing the sample's retention time with the reference material (37 Component FAME Mix, Supelco), following by the calculation of prominent fatty acids composition in the sample. The content of fatty acids was expressed as a percentage of each fatty acid.

2.1.6. Tocopherol content

Tocopherol content was determined based on the method described by [Siger et al. \(2014\)](#). A sample (200 mg) was weighed into the volumetric flask, then filled with hexane until it reached 10 mL. The mixture was vortexed thoroughly and transferred into a vial. The analysis was carried out using a Waters HPLC system (Waters, Milford, MA) equipped with a pump (Waters 600), fluorometric detector (Waters 474), photodiode array detector (Waters 2998 PDA), autosampler (Waters 2707), column oven (Waters Jetstream 2 Plus), and LiChrosorb Si 60 column (250 $\times$ 4.6 mm, 5 μ m) by Merck (Darmstadt, Germany). N-hexane and 1, 4-dioxane (96:4 v/v) were used as the mobile phase with 1 mL/min flow. The tocopherol content was determined referring to the α -, β -, γ -, and δ -tocopherol standards (>95 % purity, Merck, Darmstadt, Germany). The readings were recorded at 295 nm and the content was expressed as mg tocopherol/100 g oil.

2.1.7. Data analysis

Training data sets (68 oils pressed in-house) were used as the authentic characteristics of BSO based on the DSC melting curve and FTIR spectra. Additionally, authentic characteristics were also determined by conventional physicochemical parameters to validate DSC and FTIR models. The data from each analysis were processed into chemometrics models for the discrimination of berry oil types intended for authenticity screening in commercial samples.

The DSC melting curves were pre-treated with a baseline correction (automatic weighted least square, order = 2), FTIR spectra were pre-treated with Standard Normal Variate (SNV) and Pareto scaling, while physicochemical data were pre-treated with auto-scaling. Data from different methods were pre-treated in different ways depending on the classification performance. After pre-treatment, all data sets were analysed using Principal Component Analysis (PCA) to visualize the distribution of the samples, then followed by classification analysis using Partial Least Square Discriminant Analysis (PLS-DA). All models were validated using Venetian blinds cross-validation (10 segments). Furthermore, the optimal latent variables (LVs) were chosen according

to the global minimum of root mean square in cross validation (RMSECV). To improve the classification performance in the DSC model, X variables with Variable Importance in Projection (VIP) scores greater than 1 were chosen to develop a better model. Testing data sets (38 commercial BSO) from DSC, FTIR, and physicochemical analysis were used to evaluate the predictive performance of all chemometrics models during authentication. All analyses were performed using Solo + MIA v.9.3 software (Eigenvector Research, Manson, WA, USA). Pearson's correlation analysis was performed using OriginPro v.10.1 software (OriginLab Corporation, Northampton, MA, USA).

3. Results and discussion

3.1. Authentic characteristics of berry seed oils

The characteristics of authentic blackcurrant (BC), raspberry (RB), and strawberry (ST) seed oils that were freshly pressed in-house were analysed based on DSC melting phase transition curves, FTIR spectra, and conventional physicochemical analysis (DPPH radical scavenging activity, oxidative induction time, total tocopherol, and fatty acid composition).

3.1.1. DSC melting phase transition

DSC melting curves of cold-pressed BC, RB, and ST are shown in [Fig. 1](#). The DSC curve of BC ([Fig. 1A](#)) was characterised by four distinctive peaks, two of which were endothermic at ± -35.21 $^{\circ}$ C and ± -24.15 $^{\circ}$ C, and two minor were exothermic at ± -40.95 $^{\circ}$ C and ± -20.28 $^{\circ}$ C. BC extracted using different types of extraction, such as ultrasound-assisted ([Piasecka et al., 2024](#)) and Soxhlet extraction ([Górska et al., 2023](#)), possessed a slightly similar profile, but the peaks reported in our study were slightly shifted to a higher temperature. For example, the prominent endothermic peak of blackcurrant oils from ultrasound-assisted extraction was at ± -40.21 $^{\circ}$ C, while the peak from Soxhlet extraction was at ± -37.41 $^{\circ}$ C. Additionally, STs ([Fig. 1C](#)) that possessed two notable endothermic peaks (± -33.08 $^{\circ}$ C, ± -17.61 $^{\circ}$ C) also shared a similar peak shape but differed in the peak temperature compared to strawberry oils from Soxhlet extraction ([Górska et al., 2023](#)). These differences were not amplified by extraction methods, but rather this was caused by the heating rate used to analyse the sample. [Górska et al.](#) used a heating rate of 15 $^{\circ}$ C/min, while in our study 5 $^{\circ}$ C/min was used. Higher heating rates may not allow sufficient time for thermal equilibrium to be reached between the sample and DSC sensor, causing the position of the original peak to shift. In contrast, a lower heating rate improved the peak resolution, resulting in a more refined and detailed presence of other supporting peaks, as depicted in [Fig. 1A and C](#).

RB ([Fig. 1B](#)) exhibited three prominent peaks, two of which are endothermic peaks (± -38.55 $^{\circ}$ C, ± -20.55 $^{\circ}$ C) and one exothermic (± -28.55 $^{\circ}$ C). These authentic fingerprints share similar peak temperatures with raspberry oils reported in previous studies ([Micić et al., 2015](#); [Oomah et al., 2000](#)). Despite the similarities, slight differences such as peak height and enthalpy were observed. In the literature, the second endothermic peak was always higher than the first endothermic peak. In contrast, we found that the second endothermic peak varied in height, noting that these oils were extracted from different seed batches and possessed a different history prior to extraction. [Micić et al. \(2015\)](#) reported that the differences in the peak shape of raspberry oils were not solely affected by the proportion of fatty acid, but also polymorphism. The authors suggested that melting less-thermally stable (metastable) polymorphs caused the rearrangement and recrystallization of the triacylglycerols (TAGs) into more stable polymorphs, as indicated by the presence of peaks at higher temperatures. In RB, the presence of first endothermic peak (± -38.55 $^{\circ}$ C) can be influenced by the melting of metastable polymorphs. The melted part was then recrystallised into a more stable polymorph and was responsible for the exothermic peak (± -28.55 $^{\circ}$ C). Finally, the second endothermic peak (± -20.55 $^{\circ}$ C) is

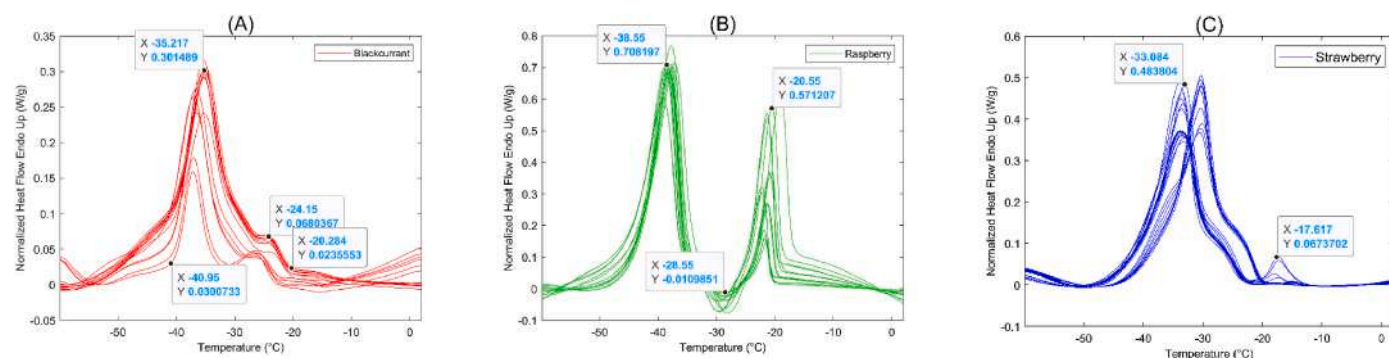


Fig. 1. DSC melting curves of cold-pressed (A) blackcurrant, (B) raspberry, and (C) strawberry seed oils, obtained at cooling rate 2 °C/min and heating rate 5 °C/min.

probably caused by the melting of the stable polymorphs. These differences observed in the peak shapes might hint at the thermal treatment history applied to seeds prior to extraction, despite being freshly pressed. Hence, variations in peak heights at higher temperatures indicate an increasing amount of stable polymorphs resulting from the repeating cycle of melting and freezing during seed storage. Through thermal fingerprints, DSCs make it possible to indicate authentic characteristics of oils, despite the variability caused by different thermal history and berry types.

3.1.2. FTIR spectra

The FTIR spectra of cold-pressed BSO at 4000 to 500 cm^{-1} are presented in Fig. 2. Different intensity of absorbances among cold-pressed BSO was observed in several spectral ranges, i.e. at 3550–3200 cm^{-1} , at 3010–2854 cm^{-1} , and at 1720–720 cm^{-1} . The variation in absorbances in the first spectral range (3600–3500 cm^{-1}) indicates the formation of oxidation products, as well as the possible presence of moisture, which corresponds to O–H stretching vibration (Embaby et al., 2022; Lim et al., 2018). Despite the absorbance variation observed in RB, the intensity of the bands in this spectral range remains relatively low, which confirms the freshness of the oils and the negligible presence of moisture. Similar results related to the low absorbance in fresh and non-oxidized edible oil at 3500–3450 cm^{-1} were previously reported by Guillén and Cabo (2002).

The spectral range at 3010–2854 cm^{-1} (Fig. 2B) mainly corresponds to the fatty acid composition of berry seed oils. The stretching of = C–H (cis) was indicated by a peak at 3010 cm^{-1} , while the rest of the peaks in this range correspond to –C–H stretching. The band intensity observed in Fig. 2B also translates into the superiority of RB in the amount of unsaturated fatty acid, followed by BC and ST, respectively. During oxidative degradation, the disappearance of the bands at 3010 cm^{-1} might be observed due to the isomerization of the double bonds from cis to trans (Valdés et al., 2015). Additionally, the ratio of peaks at 3010/2925 cm^{-1} was often used in a rapid authenticity study, as it

correlates with the ratio of unsaturated (UFA) to saturated fatty acid (SFA) (Poiana et al., 2015). In our study, it was confirmed by a significant Pearson's correlation between the ratio of UFA/SFA and peaks at 3010/2925 cm^{-1} in BSO from the training dataset ($r = 0.625$, $p\text{-value} \leq 0.05$). Higher band intensities in RB were observed in the spectral range of 1720–720 cm^{-1} . This range corresponds to the presence of free fatty acids (FFA) at 1720 cm^{-1} , the reduction in *cis* double bonds (1654 and 914 cm^{-1}), trans double bonds (968–964 cm^{-1}), as well as the presence of ketones and aldehydes from the secondary oxidation products (969 cm^{-1}) (Lim et al., 2018). A spectral range of 1800–700 cm^{-1} is often recognised as the FTIR fingerprints, hence, chemometric models to discriminate botanical origin were often built based on the absorption intensity in this region (Socaciu et al., 2020). However, no visible absorption differences between BC and ST were observed in this spectral range, except for a higher peak for strawberry oils at 1139.9 cm^{-1} . Considering these traits of each berry types, FTIR spectra demonstrated a satisfactory performance to rapidly assess the differences in quality and the composition of oils that differs in types and production batches.

3.1.3. Physicochemical properties

The physicochemical properties of BSO extracted from the various fruits' seeds are presented in Table 1. Among the samples, RB possessed the highest DPPH radical scavenging activity. This characteristic was followed by the highest amount of total tocopherol, as well as the oxidative stability as indicated by the oxidation induction time (OIT) at 120 and 140 °C. RB contained the highest total amount of unsaturated fatty acid, followed by BC, and ST. These results confirmed the previous discussion about the fatty acid composition projected in FTIR spectra (Fig. 2B). Furthermore, RB were characterized by its significant amount of linoleic acid (c18:2), but less oleic acid (c18:1) compared to other samples. Our recent works reported the relationships between DSC melting profile parameters and fatty acid compositions of raspberry seed oil. For example, the peak temperatures were positively correlated with c18:1 and c18:2, and negatively correlated with triunsaturated fatty

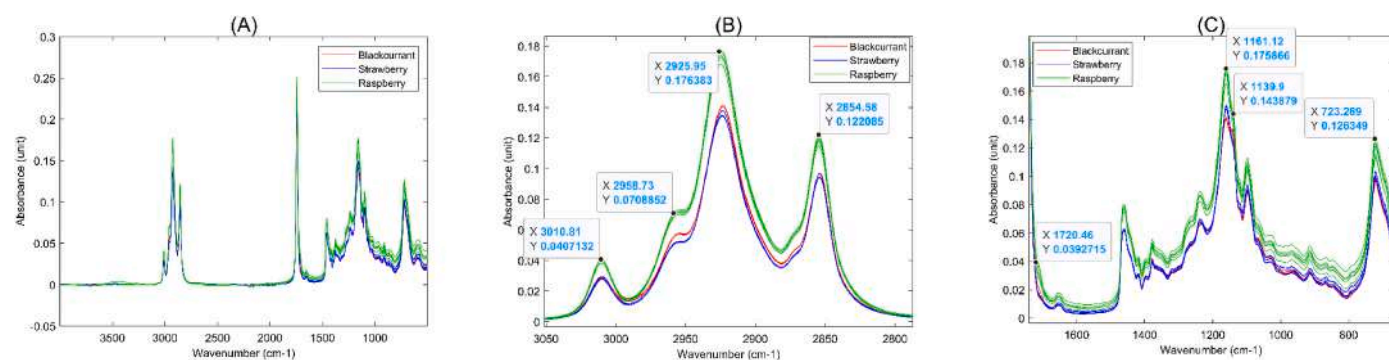


Fig. 2. FTIR spectra of cold-pressed blackcurrant, raspberry, and strawberry seed oils (A) ranging from 4000 to 500 cm^{-1} , (B) ranging from 3050 to 2800 cm^{-1} , (C) ranging from 1730 to 700 cm^{-1} .

Table 1
Physicochemical properties of berry seed oils.

Dataset	Parameters	BC (N = 10)		RB (N = 10)		ST (N = 10)	
		Min	Max	Min	Max	Min	Max
Training	DPPH ($\mu\text{mol TE/g oil}$)	3.89	7.46	20.90	22.83	6.00	8.30
	OIT 120 (min)	55.88	65.50	88.46	96.48	17.47	25.36
	OIT 140 (min)	14.60	18.56	23.15	25.59	4.14	5.63
	Total tocopherol (mg/100 g)	112.97	142.03	190.43	221.80	32.99	43.56
	c18:1 (%)	16.90	17.31	11.28	11.40	15.99	16.07
	c18:2 (%)	44.93	52.86	55.01	55.17	48.27	48.49
	c18:3 n-3 (%)	12.56	12.88	29.87	30.18	28.73	29.16
	c18:3 n-6 (%)	13.01	13.29	0.07	0.08	0.07	0.07
	ΣSFA (%)	3.66	12.60	3.17	3.77	6.21	6.94
Testing	DPPH ($\mu\text{mol TE/g oil}$)	1.98	4.09	15.18	17.46	1.79	4.56
	OIT 120 (min)	54.62	75.83	99.67	108.05	40.88	50.32
	OIT 140 (min)	10.99	16.56	23.14	26.59	7.69	12.48
	Total tocopherol (mg/100 g)	54.53	79.18	182.24	202.63	45.85	65.11
	c18:1 (%)	30.82	31.22	16.38	16.47	19.26	19.28
	c18:2 (%)	54.72	54.95	61.47	61.53	55.51	55.80
	c18:3 n-3 (%)	1.83	1.91	14.91	15.13	16.10	16.13
	c18:3 n-6 (%)	1.97	2.01	0.03	0.04	0.11	0.12
	ΣSFA (%)	9.91	10.66	6.83	7.21	8.67	9.02

BC: Blackcurrant oil, RB: Raspberry oil, ST: Strawberry oil, N: Number of samples, DPPH: DPPH radical scavenging activity, OIT: oxidation induction time, SFA: saturated fatty acid.

acids (c18:3 *n*-3 and *n*-6) (Rajagukguk, Islam, Grygier, & Tomaszewska-Gras, 2023).

BC can be differentiated based on the content of γ -linolenic fatty acid (c18:3 *n*-6), which is almost absent in other samples. Moreover, BC contained a significant amount of tocopherol, which contributed to its oxidative stability being higher than ST. Finally, ST was characterised by distinctive proportions of oleic, linoleic, and α -linolenic (c18:3 *n*-3) fatty acid. Despite having the lowest amount of tocopherol, it exhibited a similar range of DPPH radical scavenging activity as BC. With respect to these distinctive characteristics, conventional analyses confirmed the validity of DSC and FTIR observations. Hence, it is necessary to explore the methodological feasibility of both instruments for rapid screening, noting that the instruments can reduce workload and time for

conventional analyses.

3.2. Exploratory analysis

Principal component analysis (PCA) was performed on raw DSC melting phase transition curve and FTIR spectra (Fig. 3) of 68 BSO. The unsupervised analysis on the full spectrum of the DSC profile was able to capture 88.43 % of total variances from the two first components (Fig. 3A). There is also a clear clustering trend based on the berry types, despite the variability of scores observed in the RB group. The main variables that explain the difference between groups, according to PC1 loading (Fig. 3B), are endothermic peaks at -38.01°C and -21.08°C , as well as an exothermic peak at -28.28°C , which corresponded to the

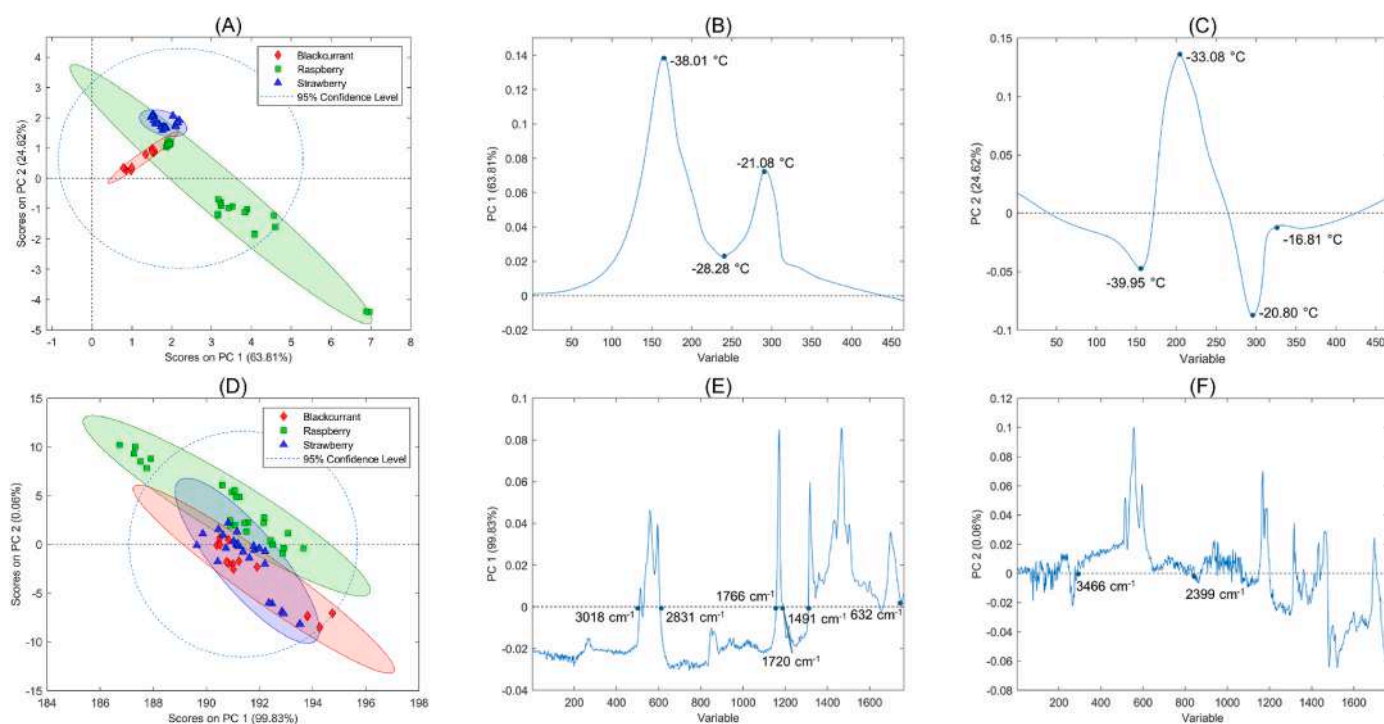


Fig. 3. PCA analysis (A) Scores plot from DSC melting profiles, (B) Loadings plot for PC1 and (C) Loadings plot for PC2. (D) Scores plot for FTIR spectra (E) Loadings plot for PC1 and (F) Loadings plot for PC2.

authentic fingerprints of raspberry oils. On the other hand, variables captured in PC2 (Fig. 3C) explain the differences between BC and ST. The trends were explained by a positive loading from the ST peak at -33.08°C and peaks on negative loadings i.e. two exothermic peaks of BC (-39.95°C and -20.80°C) and a minor endothermic peak of ST (-16.81°C). These grouping trends indicate the different distribution of fatty acids for each oil type, as marked by the peak temperatures in the DSC melting curve. Contrary to the previously reported method using specific DSC parameters (peak temperature, height, and enthalpy) during authenticity assessment (Sanahuja et al., 2011; Tomaszewska-Gras, 2016a), the full spectrum of DSC melting curves was used in this study. This approach was proven to be time-effective and suitable for qualitative observations, as it provides credible information about the grouping trends.

In the case of FTIR spectra, the first two components of PCA were able to capture 99.89 % of total variances in (Fig. 3D). PC1 loading (Fig. 3B) presents some dominant variables that positively influence the group separation such as the absorbance at $3018\text{--}2831\text{ cm}^{-1}$, $1766\text{--}1720\text{ cm}^{-1}$, and $1491\text{--}632\text{ cm}^{-1}$. These spectra correspond to the fatty acid composition and oil quality, respectively, as discussed in Chapter 3.1.2. Additionally, PC2 loading (Fig. 3D) also confirmed the maximized variances observed at $3466\text{--}2399\text{ cm}^{-1}$ that were positively contributed to the separation between groups. Despite the high percentage of variance captured, it does not reflect a meaningful separation between berry types. It implies the necessity to process the data with another alternative method designed to maximize group separation with regard to the class labels, such as PLS-DA. Considering the high amount of variance attributed to negative loading in both PCs, it is necessary to reduce potential noise in order to maximize the differences between groups. Overall, the FTIR technique offers a promising capability to rapidly identify berry oil types based on fatty acid composition and

quality state. However, it requires a proper data processing method to extract useful information from the model.

3.3. Classification analysis

Prior to the development of the authentication model, two popular classification methods were explored, which are PLS-DA as discriminant analysis and SIMCA as a one-class classifier. Both methods possessed different purposes and principles. PLS-DA aimed to establish boundaries between classes and to determine whether testing data belong to any of the pre-defined classes. On the other hand, SIMCA aimed to establish a boundary for a target class and to decide whether some testing data belong to one target class or are not classified at all. In a hard version of PLS-DA, the problem arises when unclassified samples are forced to be allocated in one of the pre-defined classes. However, this problem can be solved by incorporating a discrimination model with outlier detection, resulting in a soft version of PLS-DA. In a food authentication study, the use of soft PLS-DA models was recently reported to detect frauds in bovine meat based on mid-infrared spectroscopy fingerprints (Nunes et al., 2020). Additionally, the authors also reported the pros and cons of using SIMCA in their dataset. For example, it was able to detect adulterated sample but performed poorly in classifying non-adulterated objects. In agreement with Nunes et al. (2020), in our study, a one-class SIMCA model was not effective for classifying berry oil types. During external validation, none of the samples were classified by SIMCA as an authentic BSO, despite its true identity. Such results were obtained mainly because of the complexity of oil matrices providing great variabilities against the initial model from the DSC melting profile and FTIR spectra. Given these circumstances, PLS-DA was utilised in the classification analysis, with the possibility of assigning unclassified samples to a separate new class.

Table 2

Results of PLS-DA models from DSC and FTIR, before and after VIP selection to discriminate genuine cold-pressed berry seeds oil types.

Data	Pre-processing	LVs (explained variance, X)	RMSECV	R ² CV	F1 score	Confusion matrices (CV, strict threshold 0.5)			
DSC curve	Baseline correction	5 (98 %, 68x465)					Blackcurrant	Raspberry	Strawberry
			0.24	0.65	1.00	Blackcurrant	16	–	–
			0.28	0.66	0.91	Raspberry	–	22	–
			0.27	0.68	0.98	Strawberry	–	–	25
						Unassigned	–	4	1
						SENS	1	0.85	0.96
						SPEC	1	1	1
	Baseline correction, VIP selection	6 (99.04 %, 68x261)					Blackcurrant	Raspberry	Strawberry
			0.23	0.62	0.97	Blackcurrant	16	1	–
			0.28	0.63	0.96	Raspberry	–	24	–
			0.16	0.88	1.00	Strawberry	–	–	26
						Unassigned	–	1	–
						SENS	1	0.92	1
						SPEC	0.98	1	1
FTIR spectra	SNV, Pareto scaling	4 (99.94 %, 68x1762)					Blackcurrant	Raspberry	Strawberry
			0.21	0.80	0.94	Blackcurrant	25	–	2
			0.13	0.89	1.00	Raspberry	–	16	–
			0.23	0.76	0.96	Strawberry	–	–	24
						Unassigned	1	–	–
						SENS	0.96	1	0.92
						SPEC	0.95	1	1
	SNV, Pareto scaling, VIP selection	6 (99.98 %, 68x883)					Blackcurrant	Raspberry	Strawberry
			0.12	0.93	1.00	Blackcurrant	26	–	–
			0.13	0.89	1.00	Raspberry	–	16	–
			0.16	0.88	1.00	Strawberry	–	–	26
						Unassigned	–	–	–
						SENS	1	1	1
						SPEC	1	1	1

SNV: standard normal variate, RMSE: root mean square error, R²: determination coefficient, CV: cross-validation, LVs: latent variables, SENS: sensitivity, SPEC: specificity.

Partial Least Square Discriminant Analysis (PLS-DA) was performed to discriminate oils based on berry types, the model performance is presented in Table 2. This table highlights the improvements of model after selecting VIP variables in both DSC and FTIR datasets. The model consisted of three individual classes (blackcurrant, raspberry, strawberry) and was created based on the pre-processed raw data.

For the DSC melting profile, a baseline correction was applied to remove the instrumental noise and enhance the data quality. The first DSC model captured nearly all the variability in the dataset (98 %, 5 LVs), and was able to explain 65 %–68 % of the variance in dependent variable (R^2 CV) with moderate RMSECV ranging from 0.24 to 0.28. This means that approximately 24 %–28 % of the oils might be incorrectly classified by the model based on cross-validation process. The first classification model by DSC achieved F1 scores ranging from 0.91 to 1.00. Although this hints at strong model performance to distinguish three classes, slight variations in F1 scores suggest the tendency of the current model to perform better for strawberry and blackcurrant classes. The highest sensitivity and specificity were denoted by 1, while the lowest were marked by 0. In agreement with F1 scores, the first model has a lower sensitivity to classify RB (SENS = 0.85), because four RB remained unassigned to any group by the validation model. In contrast, decent classification performance of BC and ST was observed.

The second DSC model only involved X variables with VIP scores greater than one, which are variables that contributed significantly to group discrimination. This treatment significantly improved the classification performance for ST, marked by a decrease in misclassification error from 0.27 to 0.16, as well as an increase in R^2 from 0.68 to 0.88. The sensitivity to classify RB was increased to 0.92, while the classification performance in BC remained similar. Additionally, improvements in the classification performance across three classes were marked by better F1 scores (0.97–1.00).

Raw FTIR data were pre-processed using SNV and Pareto scaling to mitigate issues related to scatter, variability, and baseline noise. This step immediately enhanced the interpretability and model performance compared to the result in unsupervised analysis. The first model captured 99.94 % variability in the data sets by 4 LVs, with R^2 ranging from 0.76 to 0.80. It shows a decent classification performance during cross-validation, as indicated by high scores for F1 (0.94–1.00), sensitivity, and specificity in all oil types. After the selection of VIP variables, the performance of FTIR models was improved. It is indicated by the lower RMSECV, perfect F1 scores, and sensitivity and specificity in classifying each oil type.

Previous studies reported similar approaches on selecting VIP scores in PLSR models to quantify the presence of adulterant based on DSC and FTIR data (Gonzalez-Ortega et al., 2024; Wang et al., 2022). Additionally, Rozali et al. (2023) presented an optimised OPLS-DA model after personalised data pre-processing and VIP selection to determine geographical and temporal origins. Unlike commercial oil screening, the aforementioned studies developed chemometrics models based on their prior information about the testing data (type and concentration of adulterant, geographical origins, harvesting seasons, etc). In a real-world scenario, the only information which buyers can obtain relates to “cold-pressed” and “berry types” claims attached to the label. Most of DSC and FTIR authentication studies started from the lab-designed problem and later developed and adjusted to real-world conditions. While such approaches are reasonable for widely studied oils and fat, they are not time-efficient for less studied oil matrices. Considering the growing market for BSO, the relevant approach to solving the emerging problem is to immediately establish reliable screening methods to authenticate the marketed oils. In this study, the factors affecting discrimination were selected based on VIP scores in the PLS-DA model. This method is commonly applied to metabolomics data to determine the most important metabolites for group discrimination (Becchi et al., 2024), which is rarely applied to DSC data in untargeted analysis. Besides developing suitable screening techniques, the greater aim of this authenticity screening is to conduct a follow-up study based

on commercial oil profiles. The limitations of our methods presented here would be the lack of comprehensive training data that can cover all the possible variabilities in DSC and FTIR profiles observed in commercial oils, as our scope is limited to cold-pressed oil.

Summing up, PLS-DA models performed well in classifying berry oil types based on DSC melting curves and FTIR spectra. The classification performance improved when relevant pre-processing was applied to each dataset. This chapter highlighted the importance of finding the most suitable pre-processing strategies from berry oils fingerprints. Additionally, the methods also hinted the potential application for other oils with similar thermal and spectral characteristics as BSO.

3.4. Authenticity screening in commercial samples

The performance of the resulting models was then tested in 38 commercial samples, consisting of 10 blackcurrant, 18 raspberry, and 10 strawberry oils (Table 3). Table 3 presents models from DSC and FTIR, as well as a model from physicochemical analysis intended to be used for validation. The X variables in the physicochemical analyses model are DPPH radical scavenging activity, OIT, tocopherol, and fatty acid composition. This model is able to classify all oils according to the proper berry types as written on the label of commercial samples. It is indicated by the perfect scores of F1, sensitivity and specificity, as well as the moderate value of RMSEP (0.18–0.28). Excluding the percentage of fatty acids, most of the minimum and maximum values in the physicochemical properties of the testing data are still within the range of training data (Table 1). Hence, the decent classification performance in this model was most likely influenced by the data variation in testing data. Regardless of the classification performance, collecting X variables used in physicochemical analyses model required considerable time, effort and materials. A practical approach to replacing these analyses would involve finding appropriate analytical instruments for rapid screening.

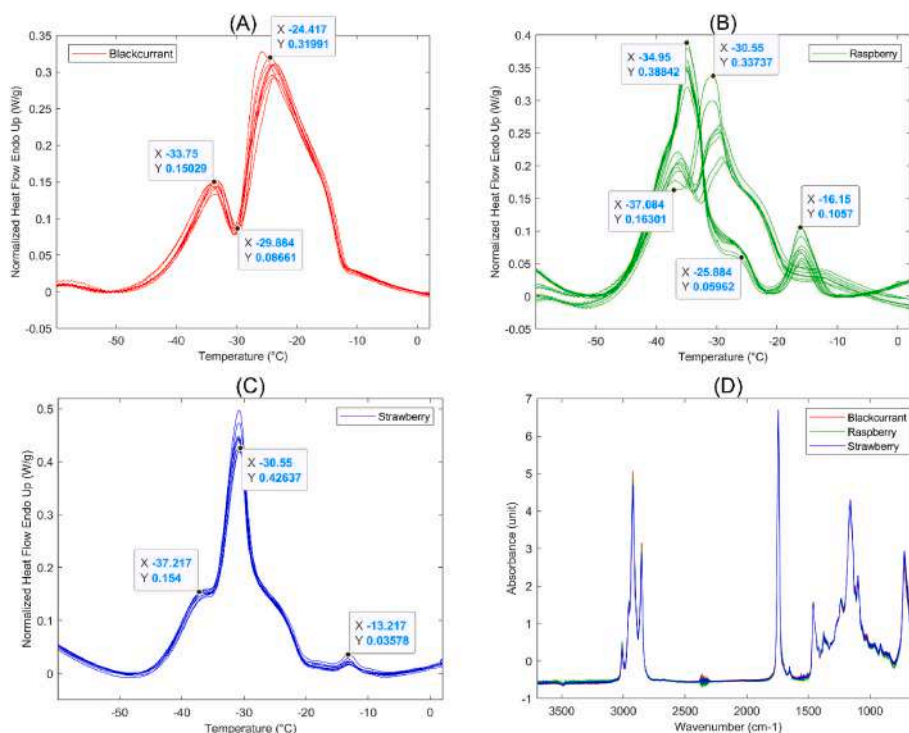
The DSC melting curves and FTIR spectra of commercial BSO, depicted in Fig. 4, were used as testing data. The model built on the DSC data showed a decent performance in classifying ST and BC. Despite having a relatively low specificity for BC classification (0.42), the model was able to classify all samples correctly according to pre-defined class. Low specificity might be caused by the big differences observed in the peak heights of commercial BC (Fig. 4A) compared to freshly cold-pressed BC (Fig. 1A). This is particularly visible at peak temperature $-24\text{ }^{\circ}\text{C}$, where the peak height for cold-pressed oils was at $\pm 0.07\text{ W/g}$ (Fig. 1A) and at $\pm 0.3\text{ W/g}$ (Fig. 4A) for commercial oils. Nevertheless, the peak temperatures of both training and testing data for BC oils were at similar values i.e. $\pm -34\text{ }^{\circ}\text{C}$ for the first peak and $\pm -24\text{ }^{\circ}\text{C}$ for the second peak.

In contrast, none of the commercial RB samples were classified into a pre-defined RB group, as it is marked by a zero score in sensitivity with a 62 % misclassification error. This poor classification performance is indicated by a zero score in F1, meaning that the model that was developed failed to identify any true positives from the commercial samples. A better F1 score could be achieved if the commercial samples match the characteristics of pre-defined RB class. Since the model classified 16 RB samples as BC and 2 RB samples as ST, this indicates a possible similarity between the DSC melting curves of commercial RB and both oils (BC and ST). Looking back to its distinguishing characteristics (Fig. 1B), RB mainly differs in the presence of two endothermic peaks at $\pm -38.55\text{ }^{\circ}\text{C}$ and $\pm -20.55\text{ }^{\circ}\text{C}$, as well as an exothermic peak at $\pm -28.55\text{ }^{\circ}\text{C}$. In contrast to the DSC melting profiles in the training set, commercial RB contained several peaks that resemble BC and ST, such as endothermic peaks at $-34.95\text{ }^{\circ}\text{C}$ and $-30.55\text{ }^{\circ}\text{C}$, respectively. In this case, the DSC model demonstrated a decent performance in recognizing the differences in the melting curves of commercial RB and assigning it to the most similar-looking group. Other than berry types, no further information about quality in commercial BSO was available, which limits the ability of this work to explain the cause of differences in

Table 3

Results of PLS-DA predictions from DSC, FTIR, and physicochemical analysis for rapid authenticity screening of commercial berry seeds oils.

Data	LVs (explained variance, X)	RMSE P	R ² P	F1 score	Confusion matrices (Prediction, strict threshold 0.5)			
Physico-chemical parameters	3 (91.87 %, 38x7)					Black-currant	Rasp-berry	Straw-berry
		0.18	0.93	1.00	Blackcurrant	10	–	–
		0.24	0.80	1.00	Raspberry	–	18	–
		0.28	0.61	1.00	Strawberry	–	–	10
					Unassigned	–	–	–
					SENS	1.00	1.00	1.00
					SPEC	1.00	1.00	1.00
DSC curve	6 (99.04 %, 38x261)					Black-currant	Rasp-berry	Straw-berry
		0.36	0.63	0.55	Blackcurrant	10	16	–
		0.62	0.66	0.00	Raspberry	–	–	–
		0.29	0.70	0.91	Strawberry	–	2	10
					Unassigned	–	–	–
					SENS	1.00	0.00	1.00
					SPEC	0.42	1.00	0.92
FTIR spectra	6 (99.98 %, 38x883)					Black-currant	Rasp-berry	Straw-berry
		0.33	0.81	0.91	Blackcurrant	10	–	–
		0.38	0.68	0.89	Raspberry	–	16	–
		0.27	0.59	0.67	Strawberry	–	2	6
					Unassigned	–	–	4
					SENS	1.00	0.80	0.60
					SPEC	0.93	1.00	0.93

RMSE: root mean square error, R²: determination coefficient, P: prediction, LVs: latent variables, SENS: sensitivity, SPEC: specificity.**Fig. 4.** DSC melting curves of commercial berry seed oils (A) blackcurrant (B) raspberry, and (C) strawberry seed oils and (D) FTIR spectra of commercial berry seed oils.

commercial RB melting curves. In the case of commercial ST oils, all samples were classified into a pre-defined ST group, as marked by a 1 score in sensitivity and 0.92 in specificity. This is justified by the clear similarity in DSC curves between cold-pressed ST (Fig. 1C) and commercial ST (Fig. 4C).

To explain the possible cause of variability in DSC curves and FTIR spectra, the relationships between parameters in physicochemical, thermal, and spectral analyses were investigated (Fig. 5). Melting peak

temperatures (Tm1, Tm2) and peak heights (hm1, hm2) were obtained from each endothermic peak in BSO, while wavenumbers 2924, 1739, 1458, and 1172 cm⁻¹ were chosen based on the greatest VIP scores. The correlation analysis was performed using parameters in testing and training data.

The peak temperature (Tm) and peak height (hm) of DSC melting curves are known to be influenced by fatty acid composition, while significant strong correlations with Tm were particularly observed for

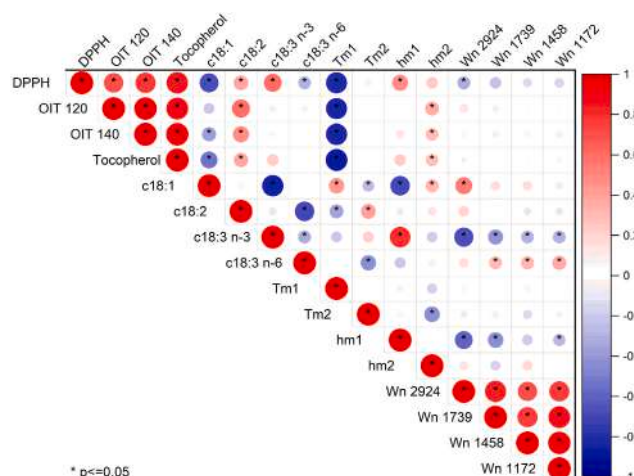


Fig. 5. Pearson's correlation between physicochemical, DSC, and FTIR parameters. Wn - wavelength number, Tm - melting peak temperature, hm - peak height.

SFA ($r = 0.787$, p -value < 0.0001) and UFA ($r = -0.788$, p -value < 0.0001) (Gila et al., 2015). The authors studied the thermal and physical properties of 14 virgin olive oils varieties and reported the natural variability in fatty acid composition and DSC curves. In agreement with the previous study, significant correlations were observed between DSC parameters (Tm, hm) with fatty acids composition (c18:1, c18:3 n-3), especially for parameter hm1 (Fig. 5). This may suggest that the most unsaturated fatty acid (c18:3 n-3) has the greatest impact on the intensity of the first melting peak (hm1), which appeared at lower temperatures. Moreover oils with a higher peak height (hm1) tend to have lower content of c18:1. In contrast to the 'varieties' aspect, processing methods were reported as not significantly affecting the fatty acid composition. A recent study by Ispiryani et al. (2023) showed that fatty acid composition of raspberry seed oil variety 'Polka' is similar when being extracted using cold-press, hexane, and supercritical extraction. Omitting the 'processing methods' aspect, it can be said that the DSC curves variability in commercial oils might be caused by different seed varieties. Other than varieties, fatty acid compositions can also be altered by adulterants, which opens up new prospects for future studies. Summing up, DSC melting curves combined with chemometrics demonstrated significant capabilities for use as rapid screening to differentiate berry oil types. To achieve optimal classification performance, it is necessary to build a model covering natural variability in BSO thermal profiles, particularly for commercial BSO with varying melting curve shapes.

The FTIR model demonstrated satisfactory performance in classifying commercial oils according to berry types. BC and RB were excellently classified, indicated by high scores for sensitivity and specificity (> 0.8) for both oils. The sensitivity score to classify ST was moderate, however, as 4 out of 10 ST remained unassigned to any of the pre-defined classes. In contrast to the cross-validation model (Table 2), which revealed a high score of R^2 in ST (0.88), the prediction model only covered a modest variance in the dependent variable ($R^2 = 0.59$). Hence, substantial unclassified ST might be caused by the variability in the FTIR spectra found in commercial samples, resulting in worse classification performance (F1 score = 0.67). Unlike DSC melting profiles, which require an additional step in data processing to identify the unassigned samples, raw FTIR spectra can be processed directly. This is because the spectra of BSO were only different in the absorbance intensity in a specific spectral range (Fig. 4D), not in the variability of shapes, as in DSC melting curves. Variations in the FTIR spectra of commercial oils might be related to different fatty acid compositions caused by seed varieties as previously discussed. It is also confirmed by the significant correlations between wavenumbers (2924, 1739, 1458, and 1172 cm^{-1})

and fatty acid composition (c18:1, c18:3 n-3, c18:3 n-6). When necessary, unclassified samples based on FTIR spectra can be processed further using clustering analysis such as Hierarchical Cluster Analysis (HCA) and K-means clustering. Recent studies reported reliable methods to process FTIR spectra with clustering analysis to ensure the authenticity of food, such as the application of HCA to differentiate high-dose irradiated hazelnuts (Dogan et al., 2020), and K-means clustering to differentiate tea varieties (Zhou et al., 2020).

Ultimately, both instruments demonstrated significant capabilities for rapid authenticity screening to differentiate oils according to berry type. Minor flaws in the prediction models from both instruments can be improved by preliminary data acquisition and other data classification techniques as alternatives. Despite the shortcomings, both instruments were able to save a great deal of time required for conventional analyses, which benefits a fast-paced analysis routine in food industry.

4. Conclusions

Both DSC melting curves and FTIR spectra captured the unique characteristics of each BSO types as observed in distinctive thermal fingerprints and different absorption intensity in certain FTIR spectral ranges. Full DSC melting curves can be used directly for qualitative observations and exploratory analysis, as they showed the grouping trends between BSO, whereas raw FTIR spectra required proper data processing techniques to obtain meaningful information about data trends. When personalised pre-processing was applied to both DSC and FTIR data, classification performance improved significantly. During authenticity assessment using commercial samples, models from both techniques exhibited decent classification performance next to the conventional physicochemical analysis. The DSC model was sensitive to recognizing the shape differences in melting curves, whereas the FTIR model was sensitive to spectrum variability, as it classified commercial samples with high variability into an unassigned class. These limitations could be overcome by building a model containing a wider range of thermal and spectral variability, not only based on freshly pressed samples, but also authentic samples of different quality and using methods of extraction. Addressing the unassigned samples, additional clustering analysis can be applied according to the study needs. Being time-efficient and reliable, both instrumental techniques can replace conventional analysis for assessing the authenticity of berry seed oils. However, further work is needed to explore more chemometric methods.

CRediT authorship contribution statement

Yolanda Victoria Rajagukguk: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jolanta Tomaszewska-Gras:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Ethical statements

This study did not include any human subjects and animal experiments.

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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Abbreviations

Analytical instruments

DSC	Differential scanning calorimetry
FTIR	Fourier transform Infrared
GC	Gas chromatography
HPLC	High-pressure liquid chromatography
MS	Mass spectrometry

Data analysis

PLS	Partial least square
DA	Discriminant analysis
O-(PLS-DA)	Orthogonal-(...)
LDA	Linear discriminant analysis
PCA	Principal component analysis
SMLR	Stepwise multiple linear regression
Bi-(PLS)	Back interval-(...)
LV	Latent variable
RMSE	Root mean square
(RMSE)-CV	(...)-Cross validation
(RMSE)-P	(...)-Prediction
VIP	Variable importance in projection
SIMCA	Soft independent modelling by class analogy
HCA	Hierarchical cluster analysis

Samples

BSO	Berry seed oils
BC	Blackcurrant seed oils
RB	Raspberry seed oils
ST	Strawberry seed oils

Compounds

FA	Fatty acid
UFA	Unsaturated fatty acids
SFA	Saturated fatty acids

Others

OIT	Oxidation induction time
Tm	Melting peak temperature
hm	Melting peak height

Data availability

Data will be made available on request.

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Fusion of DSC and FTIR data with physicochemical profiling to distinguish berry seed oils by extraction methods

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Abstract:	Growing concerns over the use of n-hexane in oil extraction, mainly due to its neurotoxicity, have led the European Food Safety Authority (EFSA) to recommend re-evaluating its regulatory limits in food. This highlights the need for reliable authentication methods. In this study, a novel data fusion approach combining differential scanning calorimetry (DSC) melting curves and Fourier transform infrared (FTIR) spectra was proposed for the rapid authentication of berry seed oils by extraction methods. The effects of three extraction techniques: cold pressing, n-hexane, and supercritical CO₂, on the physicochemical, thermal, and spectral profiles of blackcurrant, raspberry, and strawberry seed oils were evaluated. Distinctive FTIR features were observed at wavenumbers 2931, 1726, 1408, and 1046 cm⁻¹, while the discriminatory DSC features corresponded to the peak temperatures. SIMCA classification models based on low-level DSC-FTIR data fusion were developed to distinguish 'oil types and extraction methods' at one run. The models showed promising performance with 81% overall accuracy in calibration and 77% in validation, which was higher than for single-method models i.e. FTIR (68%, 67%) and DSC (65%, 64%). These results demonstrated the potential of this method for rapid oil screening, supporting authentication protocols for regulatory compliance and label transparency in food industry
Response to Reviewers:	Dear Reviewers, Thank you very much for taking the time and effort to help us improve the quality of our manuscript. We sincerely appreciate your insightful comments and suggestions, all of which we have carefully considered and addressed.

Poznań, September, 25, 2025

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Scientific Handling Editor

Raffaele Colosimo, PhD

Journal of Food Composition and Analysis

Dear Raffaele,

Thank you very much for giving us the opportunity to revise our manuscript with a new title: *“Fusion of DSC and FTIR data with physicochemical profiling to distinguish berry seed oils by extraction methods”*

We have carefully considered and addressed all comments raised by the Reviewers. We are grateful to the Reviewers for their valuable comments and suggestions, which have helped us improve the quality of the manuscript. Every change made in response to the Reviewers' comments has been clearly outlined. In the revised version, all corrections and new additions are highlighted in red.

We hope that the revised manuscript now meets the standards for publication in the *Journal of Food Composition and Analysis*.

Yours sincerely,

A handwritten signature in blue ink that reads "Jolanta Gras". The signature is written in a cursive, flowing style.

Jolanta Tomaszewska-Gras

Fusion of DSC and FTIR data with physicochemical profiling to distinguish berry seed oils by extraction methods

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Abstract

Growing concerns over the use of *n*-hexane in oil extraction, mainly due to its neurotoxicity, have led the European Food Safety Authority (EFSA) to recommend re-evaluating its regulatory limits in food. This highlights the need for reliable authentication methods. In this study, a novel data fusion approach combining differential scanning calorimetry (DSC) melting curves and Fourier transform infrared (FTIR) spectra was proposed for the rapid authentication of berry seed oils by extraction methods. The effects of three extraction techniques: cold pressing, *n*-hexane, and supercritical CO₂, on the physicochemical, thermal, and spectral profiles of blackcurrant, raspberry, and strawberry seed oils were evaluated. Distinctive FTIR features were observed at wavenumbers 2931, 1726, 1408, and 1046 cm⁻¹, while the discriminatory DSC features corresponded to the peak temperatures. SIMCA classification models based on low-level DSC-FTIR data fusion were developed to distinguish ‘oil types and extraction methods’ at one run. The models showed promising performance with 81% overall accuracy in calibration and 77% in validation, which was higher than for single-method models i.e. FTIR (68%, 67%) and DSC (65%, 64%). These results demonstrated the potential of this method for rapid oil screening, supporting authentication protocols for regulatory compliance and label transparency in food industry.

Keyword: blackcurrant, raspberry, strawberry, data fusion, solvent extraction, hexane, cold pressing, supercritical CO₂, chemometrics

1. Introduction

Increased in berry seed oil production and consumption have been observed in recent years due to its economically lucrative aspect and its health promoting properties (Przykaza et al., 2021). Edible oils are commonly extracted through cold pressing, solvent, and supercritical CO₂ extraction (Aytaç, 2022). All three techniques may result in different oil quality and safety parameters. For example, cold-pressed berry seed oils are receiving greater attention due to their bioactive properties and suitability for direct consumption. Supercritical CO₂ extraction gained popularity with its green technology and low-temperature processing. This results in premium oil quality with volatile aromas preserved (Moreira et al., 2023). Meanwhile, solvent extraction is often chosen by producers for its operational efficiency, broad compatibility in any seed matrices, and relatively higher yield than cold-pressing extraction. In the food industry, n-hexane is used extensively to extract various fats and oils. However, the use of n-hexane in food production sparked some discussions in recent years, mainly because of its neurotoxicity and the issue of food label clarity, as mentioning labelling of “hexane-extracted products” is not mandatory according to current EU law (Cravotto et al., 2022).

Following the growing concerns, European Food Safety Authority (EFSA) addressed the need to re-evaluate the safety aspects of hexane as a solvent in food production due to its toxicological adverse effect and potential exposure for consumers (Comandella et al., 2024). The need to re-evaluate also emerged as the latest hexane exposure assessment conducted by the Scientific Committee on Food (SCF) in 1996 was far behind the recent scientific developments. In European Union, the maximum hexane residue in fat or oil, which is 1 mg/kg product (European Commission, 2009), was controlled by Reg. EU 2009/32/EC. EFSA assessment concluded that the current regulatory limit might be too high because the maximum mean of actual dietary exposure in humans is much lower than the regulation limit, especially for infants and toddlers. This indicates the need to set a new standard for maximum hexane residue levels for food products. Beyond the regulatory scale, this issue also implies the need for researchers and practitioners to seek for alternatives in consumer health protection. Therefore, in this work, we propose a chemometric method with low-level data fusion to swiftly classify berry seed oil types and their extraction methods, namely cold pressing, solvent extraction by n-hexane, and supercritical CO₂ extraction.

Data fusion is the integration of two or more datasets obtained from different analytical instruments into one single array, with the aim being to enhance the characterisation, classification, and even prediction of samples. This technique is divided into three levels, which are low-, mid-, and high fusion. Low-level fusion combined directly both pre-processed

datasets, mid-level fusion extracted the features from each datasets based on a variable selection procedure then combined these data into one, while high-level fusion combined the models' decision from different datasets (Hong et al., 2023). For more than a decade ago, the application of data fusion in food authentication studies has increased steadily. The reported works mainly combine spectroscopic and chromatographic data from various food matrices (Borràs et al., 2015; Callao and Ruisánchez, 2018). Various analytical instruments were combined due to their abilities to complement each other's performance to analyse sample properties.

Previous works reported the complementary usage of both differential scanning calorimetry (DSC) and Fourier transform infrared (FTIR) spectroscopy to investigate the effect of different extraction methods on thermal and spectral properties in black cumin and yellow horn seed oils (Albakry et al., 2023; Gu et al., 2019). Both studies suggested the influence of extraction methods on absorption intensity in a specific FTIR spectral range. However, the effect on DSC thermal properties was rather inconclusive. For example, a slight deformation of the melting curves occurred in black cumin seed oil without affecting its peak temperature. Additionally, our preliminary works demonstrated the individual feasibility of DSC and FTIR for the rapid authenticity screening in berry seed oils (Rajagukguk and Tomaszewska-Gras, 2025). The feasibility was achieved because DSC can rapidly characterise berry seed oils based on thermal fingerprints associated with its fatty acid composition, while FTIR was able to distinguish berry seed oils' authenticity based on specific spectral intervals.

While previous works reported the individual feasibility of DSC and FTIR, the integration of both datasets remains underexplored for the discrimination of extraction methods. It represents a significant research gap, noting that single-technique approaches may miss key discriminatory information for rapid authentication by chemometrics. Additionally, thermal analysis was poorly represented in the general scope of the data fusion framework. Such conditions are considered counterproductive with regard to DSC feasibility in authenticity studies. To best of our knowledge, this work is the first attempt to investigate whether data fusion of DSC and FTIR can overcome the limitations from individual techniques to distinguish berry oil types and extraction methods at one run. Hence, we hypothesise that thermal and spectral data would complement each other by low-level data fusion, leading to better classification performance compared to single analytical techniques.

The first aim of this study was to investigate the influence of different extraction methods (cold pressing, n-hexane, and supercritical CO₂ extraction) on physicochemical, thermal and spectroscopic profile of berry seed oils (blackcurrant, raspberry, and strawberry).

Secondly, it aimed to explore the relationship between conventional quality analyses and analytical instruments (DSC, FTIR). Finally, the broader aim of this work was to develop a reliable analytical technique for rapid classification of berry oil types in terms of extraction methods, which may benefit practitioners in the oil industry during procurement screening.

2. Material and methods

2.1 Materials

Berry seeds, namely blackcurrant (*Ribes nigrum*), raspberry (*Rubus idaeus*), and strawberry (*Fragaria ananassa*), were purchased from GreenField Sp. z.o.o (Warsaw, Poland). Each berry seed type was purchased from three different production batches to capture the natural variability of samples. A total of nine sets of seeds were extracted using the cold pressing-, solvent-, and supercritical CO₂ techniques. Prior to extraction, the seeds were stored in a vacuumed aluminium bag at -20 °C in the dark.

2.2 Methods

2.2.1 Extraction of berry seed oils

To obtain the cold-pressed berry seed oils, the seeds were pressed directly using a kitchen-scale oil presser (Counter Intelligence Oilpresso, Netherlands) with the default temperature at 40 °C. The crude pressed oils were then filtered to remove the seed debris and kept in a brown-glass bottle directly after pressing.

Prior to hexane and supercritical CO₂ extraction, the berry seeds were ground into a fine powder to ease the extraction process. Hexane extraction were conducted following the method described by Rajagukguk et al. (2022) with a slight modification. Milled berry seeds (100 g) were dissolved in n-hexane (Sigma-Aldrich, Steinheim, Germany) with a 1:2 (w/v) ratio. The mixtures were constantly stirred for 30 min, then transferred to an evaporating flask. Hexane was evaporated by a rotary vacuum evaporator (The Rotavapor® R-300, BÜCHI Labortechnik AG, Flawil, Switzerland) under the following conditions: pressure 264 mbar, 30 rpm, bath temperature 50 °C, time 30 min. Evaporation was stopped once the hexane was thoroughly evaporated. The resulting oils were then transferred into a glass vial and purged with nitrogen gas to remove the remaining hexane for ±10 min.

Supercritical CO₂ extraction was conducted following the method described by Marić et al. (2020) with a slight modification. Milled seeds (20 g) were transferred into the extraction vessel. The extraction was carried out using a multi-vessel supercritical extraction system (MV-10ASFE Waters, Manchester, MA, USA) consisting of CO₂ cylinder and a cooling system, fluid delivery module, column oven, back pressure regulator, heat exchanger and fraction

collection module with ChromScope v1.20 software (Waters, Manchester, MA, USA). The extraction parameters were set to the following conditions: pressure 325 bar, oven temperature 40 °C, make up flow 0.2 ml/min, and the duration set for dynamic 1, static, and dynamic 2 was 10, 30, and 110 min, respectively. The resulting oils were collected in glass container attached to the extractor instrument.

After each extractions, all berry seed oils ($n=27$) were stored in brown-glass bottles, purged with nitrogen gas (99.99% purity), and kept in freezer at -80 °C until analysis.

2.2.2 Physicochemical analysis

The physicochemical characteristics of berry seed oils obtained from different extractions were investigated by the following analyses: acid value (AV), p-anisidine value (p-AnV), peroxide value (PV), specific absorbance coefficient of oils at λ 232 nm and 268 nm (K_{232} , K_{268}), DPPH radical scavenging activity, colour analysis, and also compositional properties such as total carotenoid, chlorophyll, fatty acid (FA), and tocopherol. All analyses were conducted in triplicate.

Acid value analysis was carried out based on standard ISO 660 (2009), and the results were expressed in mg KOH /g oil. P-anisidine values were determined following the method described in ISO 6885 (2016), and these results were expressed in absorbance units. The peroxide value was determined based on ISO 27107 (2008) and expressed in milliequivalents of active oxygen per gram of oil (mEq O₂/g oil). The specific absorbance coefficient of oils (K-values) was investigated following the method described by AOCS Ch 5-91 (2009). K_{232} and K_{268} values were expressed in absorbance units, and these values represent conjugated dienes and trienes as the products of oil oxidation. DPPH radical scavenging activity was analysed using the method described by Sánchez-Moreno et al. (1998) and expressed in μ mol of Trolox equivalent (TE) per gram of oil.

The colour properties of oils from different extraction methods were investigated according to the CIELAB colour system (L^* , a^* , b^* , C^*_{ab} , and h_{ab}) and were presented using chroma (C^*_{ab}) and angle hue (h_{ab}) parameters. Chroma indicates the relative saturation, while angle hue represents the gradation of colour from colour wheel.

Total carotenoid content was determined using the method described by Romanić et al. (2021) and presented as the total of carotenoid in mg/g oil. Chlorophyll content was measured according to AOCS cc13i-96 (2009a) and expressed in mg of pheophytin A/g oil. Tocopherol content was determined according to method described by Siger et al. (2014), by which the content of α -, β -, γ -, δ -tocopherols, and vitamin E in the oil were calculated. Tocopherol content

was expressed in mg of tocopherol/g oil. Fatty acid composition analysis was conducted according to the AOCS Ce 1 h-05 method (2005). Quantification of fatty acid composition was referred to an internal standard, namely methyl heptadecanoate (Sigma Aldrich, USA). The results were expressed in mg/g oil.

2.2.3 DSC thermal analysis

The melting profile of berry seed oils was determined using DSC 8500 (Perkin Elmer, Waltham, MA, USA) in accordance with Tomaszewska-Gras (2016). The instrument was programmed as follows: 1) heating at 30 °C for 5 min, 2) cooling with scanning rate 2 °C/min from 30 °C to -67 °C, 3) heating at scanning rate 5 °C/min from -67 °C to 30 °C. The whole heating spectrum of DSC was utilised to build the model for the identification of oil extraction methods.

The oxidative stability of berry seed oil at 120 °C and 140 °C was measured by isothermal analysis using DSC, following the ISO 11357-6 method (2018). The programme parameters were personalised for berry seed oils (Rajagukguk et al., 2023). Oxidation induction time (OIT) was measured in duplicate and expressed in minutes (min).

2.2.4 FTIR spectroscopy analysis

A Spectrum Two ATR FTIR spectrometer (Perkin Elmer, Waltham, MA, USA) was utilised to capture the FTIR spectra of BSO from 4000 – 500 cm⁻¹ wavenumbers with 4 scans per sample at 4 cm⁻¹ resolution in reflectance mode. The resulting spectrum was wholly utilised to build the model for identifying oil extraction methods.

2.2.5 Data analysis

Chemometric workflow is visualised in Fig 1. Each DSC and FTIR dataset consists of 54 samples and both datasets were pre-processed separately. DSC melting curves were normalised by dividing the heat flow by the sample weight. Normalised heat flow was treated with baseline correction (automatic weighted least square, order = 2). FTIR spectra were pre-processed using Standard Normal Variate (SNV), mean-centring (MC), and smoothing (SavGol, filter width: 15, polynomial order: 0, derivative order: 0). Both pre-processed DSC melting profiles and FTIR spectra were fused into one dataset and subsequently addressed as “DSC-FTIR”. No further data pre-processing was applied to the fused data. These datasets were subjected to Principal Component Analysis (PCA) to visualise the distribution of score plots and the data trend exploration.

The datasets (DSC, FTIR, DSC-FTIR) were then analysed using Soft Independent Modelling by Class Analogy (SIMCA). Models were developed to classify three independent

categories, namely ‘berry types’, ‘extraction methods’, and ‘berry types – extraction methods’. Prior to SIMCA analysis, datasets were divided into calibration and external validation subsets, total samples in each subsets were depending on the independent category. For the classification of ‘berry types’ and ‘extraction methods’, datasets (n=54; 3 classes, 18 samples/class) were divided as follow: calibration (66%, n=36) and validation (33%, n=18) subsets. Calibration subsets were internally validated with 12-fold Venetian blinds cross-validation (CV). During the classification of ‘berry types – extraction method’, datasets (n=54; 9 classes, 6 samples/class) were divided by 80% for calibration (n=45) and 20% for external validation (n=9). Subsequently, calibration subsets were internally validated with 5-fold Venetian blinds CV. The representation of each class in calibration and validation was ensured during the dataset division. The developed models were then used to classify samples from validation subset. The whole process was repeated three times (for ‘‘berry types’ and ‘extraction methods’) and six times (for ‘berry types – extraction method’), with new calibration and validation subsets for each iteration. Model performance metrics (sensitivity, specificity, accuracy, F1 score) from both calibration and validation subsets were reported with 95% Clopper-Pearson confidence intervals.

Three classes in ‘berry types’ classification are blackcurrant – BC, raspberry – RB, strawberry – ST. In ‘extraction methods’, three classes consist of cold press – P, n-hexane extraction – E, and supercritical CO₂ extraction – S. Finally, there are 9 pre-defined classes (BC-P, BC-E, BC-S, RB-P, RB-E, RB-S, ST-P, ST-E, ST-S) for ‘berry types – extraction method’ classification. Data analysis was performed using Solo + MIA v.9.5 software (Eigenvector Research, Manson, WA, USA). Graph visualisation and Pearson’s correlation of physicochemical, thermal, and spectroscopic properties were conducted using OriginPro 2024 v.10.1 software (OriginLab Corporation, Northampton, MA, USA).

3. Results and discussion

3.1 Physicochemical properties as affected by oil extraction methods

Physicochemical properties of blackcurrant-, raspberry-, and strawberry seed oils extracted using cold pressing, n-hexane, and supercritical CO₂ extraction are presented in Fig. 2, which shows the average values of individual oils extracted from three different seed batches.

Among the extraction methods, supercritical CO₂ extraction enabled the highest yield to be obtained (up to 0.2 g/g), followed by n-hexane (up to 0.08 g/g), and cold pressing (up to 0.09 g/g), as shown in Fig. 2B. A recent study investigated the superiority of supercritical CO₂ to extract more yield from plant seed with smaller seed size (flaxseed, linden, poppy, marigold)

and lower oil content (linden, marigold) compared to hexane extraction and cold pressing (Schoss and Glavač, 2024). It has also been reported that factors such as seed dryness, particle size and temperature significantly influenced yields. For instance, for the supercritical CO₂ raspberry seed oil extraction, grinding seeds through a 0.5 mm sieve increased the yield by 2 % to the final level up to 19 % (Ispiryan et al., 2023). Oils from this extraction are more superior in terms of quality, as they were processed under mild temperatures without oxygen exposure. Furthermore, the oils are free from chemical residue, making them suitable for direct consumption. Despite the advantages, this method is rather expensive because of the cost of instrumentation, maintenance, and the pure CO₂ as the main solvent. Moreover, utilisation of supercritical CO₂ methods requires specific expertise during the experimental design and parameter optimisation stages (temperature, pressure, time, flow rate, and co-solvent), which greatly affects the yield and characteristics of the resulting oils (Ahangari et al., 2021).

In a comparison of berry types, strawberry oil extraction resulted in the highest yield (up to 0.2 g/g) compared to raspberry (up to 0.15 g/g) and blackcurrant oil (up to 0.12 g/g) in all extraction methods. Blackcurrant oil extraction by cold pressing produced the lowest yield (0.01 g/g). Cold-pressed blackcurrant oil possessed a thick and non-fluid viscosity, making it harder to separate from the seeds during mechanical extraction. This was caused by the abundant amount of pectin (12 g/100 g) from the blackcurrant seeds cell walls (Konrade et al., 2023). In contrast to the cold pressing technique, blackcurrant oil extracted by n-hexane and supercritical CO₂ extraction possessed a typical viscosity, as shown in raspberry and strawberry oil. Considering these striking differences in yield, the product characteristics of the resulting oil should be defined before extraction, after which the most appropriate extraction method can be selected. For example, if the producer seeks to market blackcurrant oils in the form of a soft gel, as in supplements, then it is reasonable to choose cold pressing as the method because the oil viscosity does not affect the appearance of the final product. In turn, if the producer strives for an economical way of producing edible blackcurrant seed oil, then hexane extraction might match the producer's aim. In this regard, extra attention should be given to preventing excessive hexane residues in oil intended for direct consumption (maximum 1 mg hexane/kg oil), as regulated in Reg. EU 2009/32/EC (European Commission, 2009). In contrast to blackcurrant oil, the yields of raspberry and strawberry oil from cold pressing and hexane extraction were roughly at a similar percentage. Hence, oil producers should opt for cold pressing due to the food safety aspect of the end products.

The quality parameters of berry seed oils that indicate freshness and oxidative status were also investigated using acid value (AV), p-anisidine value (p-AnV), and peroxide value

(PV), as well as specific extinction values indicating the presence of conjugated dienes (K_{232}) and trienes (K_{268}). The highest values for AV (Fig. 2A) were observed in raspberry seed oil, indicating the quality of raw materials prior to each extractions. These were around 7 - 15 mg KOH/g oil, which is higher than the CODEX quality standard at 4 mg KOH/g oil (Codex Alimentarius, 2015). In turn, for strawberry- and blackcurrant seed oils, AV values were close to this limit. Such values were expected to be found in raw berry seed oils, noting that these oils were extracted from the by-products of berry processing. Prior to extraction, the berry seeds had undergone a longer pre-processing step than other raw materials for oils. The by-products were dried after berry processing, and the seeds were then mechanically separated from pomace, stored at a temperature below zero, and thawed before extraction. Longer pre-processing time and being exposed to various extreme temperatures were proved to affect berry seed quality (Radočaj et al., 2014).

The oxidative status of oils in the primary and secondary stages was tested by analysing p-AnV (Fig. 2A) and PV (Fig. 2B). By measuring PV, the concentration of peroxide and hydroperoxide compounds is determined, while p-AnV is used to assess the quantity of 2-alkenals and 2,4-alkadienals. The highest PV and p-AnV were observed in cold-pressed oils (up to 0.033 mEq O₂/g), followed by n-hexane (up to 0.023 mEq O₂/g) and supercritical CO₂ extraction (up to 0.018 mEq O₂/g). These values are higher than 0.010 mEq O₂/g oil as regulated for cold-pressed oils (Codex Alimentarius, 2015). A higher oxidation level in cold-pressed oils compared to other solvent extractions was also reported in previous studies (Wang et al., 2022; Wongwaiwech et al., 2023). This might have been caused by air exposure during extraction, while oils from hexane and supercritical CO₂ extraction contained lower oxidation products because the process took place in closed vessels. The dominant level of oxidation products in most of the cold-pressed oils was confirmed by K_{232} and K_{268} values (Fig. 2B). Additionally, PV and p-AnV in all oils were in a similar range with the values reported by Mildner-Szkudlarz et al. (2019). Regardless of the berry type and extraction method, most of the quality parameters in berry seed oils exceed the existing regulatory standards. To meet the regulations, oil producers have no choice but to refine the resulting berry seed oils, which unfortunately could strip away the natural antioxidants present in oils (Szydłowska-Czeraniak and Łaszewska, 2015). In regard to the growing popularity of oils from by-products, developing a new regulatory standards specifically for these oil groups might be an necessary approach to address the future food safety needs.

Abundant quantities of carotenoid and chlorophyll were observed in most of the cold-pressed oils (Fig 2B). Regardless of the extraction method, blackcurrant oils possessed the

highest total carotenoid (approx. 0.11 mg/g oil) and chlorophyll content (approx. 0.35 mg of pheophytin A/g oil) compared to raspberry and strawberry oils. These values are in a similar range with the total carotenoid and chlorophyll content of blackcurrant seed oils extracted using supercritical fluid extraction at 330 bar (Wójciak et al., 2022). With respect to its abundant pigments, the appearance of blackcurrant oils (Fig 2C) was dark yellowish, as indicated by its hue (approx. 71°), chroma (approx. 15°), and L* (approx. 26.51). No notable differences were observed in total carotenoid and chlorophyll content nor in the angle hue of raspberry and strawberry oils from three different extraction methods. However, cold-pressed raspberry and strawberry oils were distinctively dark, as indicated by their respective chroma (11.74°, 1.80°) and L* values (44.44, 48.63). A dark appearance in cold-pressed oils might be caused by the presence of seed residues and other impurities. Additionally, crude raspberry oils from three methods exhibited exceptionally high values in DPPH radical scavenging activity (approx. 192 µmol TE/g oil), as shown in Fig. 2C, while the values found in commercial raspberry seed oils were reportedly lower at approx. 60 µmol TE/g oil (Luo et al., 2021). This could indicate the possible refining process conducted by the oil producer to improve oil stability in commercial oils.

Initial oil quality and the concentration of bioactive compounds were reported to affect the oxidative stability and shelf life of seed oils (Mikołajczak and Tańska, 2022). Oxidative stability was determined in our study by differential scanning calorimetry (DSC) based on oxidation induction time (OIT), which represents the time needed until oxidation occurs under a constant isothermal temperature, as depicted in Fig 2D. Cold-pressed raspberry seed oils were shown to exhibit the highest OIT (approx. 99 min) at temperature 120 °C, followed by n-hexane (approx. 90 min), and supercritical extraction (approx. 80 min). Such differences in oxidative induction time might be attributed to the darker appearance of cold-pressed oil, considering that other quality parameters possessed by these oils were at a similar level (carotenoid, chlorophyll) with other extractions, or not favouring increased stability (AV, PV, and p-AnV). Conversely, a dark appearance did not greatly affect the OIT of cold-pressed strawberry oils. Slight differences in OIT at 120 °C (approx. 5 min) and OIT at 140 °C (approx. 2 min) were found in the rest of the oils from other extractions.

Figure 2E shows the fatty acid (FA) composition. The composition of all berry seed oils was dominated by linoleic acid (c18:2), ranging from 283 mg/g for strawberry seed oil to 352 mg/g for raspberry seed oils. The FA composition of blackcurrant seed oils was distinctive with its presence of γ -linolenic acid (c18:3 *n*-6) and 1:1 ratio with α -linolenic acid (c18:3 *n*-3).

However, the highest content of α -linolenic acid was detected in raspberry seed oil (from 188 to 196 mg/g), followed by strawberry seed oil (from 174 to 177 mg/g). Similar FA proportions in blackcurrant-, raspberry-, and strawberry seed oils were also reported in other studies using cold pressing, hexane, and supercritical CO₂ extraction (De Filette et al., 2024; Ispiryan et al., 2023; Yang et al., 2011). Overall, extraction methods did not affect FA composition in the berry seed oils in this study. This result is in agreement with previous studies on different extraction methods and parameters such as particle size and temperature (Gustinelli et al., 2018; Ispiryan et al., 2023). Additionally, Ispiryan et al. (2023) noted that variations in FA composition in the same berry oil types were mainly affected by the seed variety rather than the extraction method.

Total tocopherol content of blackcurrant-, raspberry-, and strawberry seed oils was approx. 1.2, 2.2, and 0.3 mg/g oil, respectively (Fig 2F). Regardless of the extraction method, these values aligned with those of previous studies (De Filette et al., 2024; Radočaj et al., 2014; Wójciak et al., 2022). The tocopherol composition of berry seed oils was mainly dominated by γ -tocopherol, followed by α -, and δ -homologues. The lowest content of γ -tocopherol was found in all n-hexane extracted oils, while γ -tocopherol from cold-pressed and supercritical extraction was at a similar level (approx. 9 mg/100 g). Similar findings on the dominance of cold pressing and supercritical CO₂ methods to obtain higher γ -tocopherol contents in seed oils were previously reported (Gustinelli et al., 2018; Ispiryan et al., 2023; Schoss and Glavač, 2024). During storage, γ -tocopherol plays an important role in protecting the oil against heat-induced oxidation, especially after the depletion of α -tocopherol content through the storage time (Rajagukguk et al., 2024). Therefore, the tocopherol content of the resulting oils should also be considered by oil producers when selecting the extraction method, as it directly affects the storage stability of berry seed oils before it reaches consumers' hands.

Summing up, extracting berry seeds using cold pressing, n-hexane, and supercritical CO₂ methods affected most of the physicochemical characteristics of the resulting oils, except fatty acid composition. Additionally, deciding on the most suitable method to extract BSO should not be based only on economic factors, but also on the physicochemical and safety aspects of the product.

3.2 Exploratory and correlation analysis based on the DSC melting profiles and FTIR spectra

3.2.1 DSC melting profiles as affected by oil extraction methods

The DSC melting profiles of three types of berry seed oils and PCA score plot are presented in Fig 3 and Table 1. Each berry seed oil type possessed a distinctive melting profile,

marked by endothermic peaks (T_{m1} , T_{m2}). In blackcurrant seed oils, they appeared at a temperature on average of $-37\text{ }^{\circ}\text{C}$, $-27\text{ }^{\circ}\text{C}$, in and strawberry seed oils $-34\text{ }^{\circ}\text{C}$, $-19\text{ }^{\circ}\text{C}$, and in raspberry seed oils there were both endothermic ($-38\text{ }^{\circ}\text{C}$, $-22\text{ }^{\circ}\text{C}$) and exothermic ($-30\text{ }^{\circ}\text{C}$) peaks. Generally, different extraction methods did not greatly affect the shape of DSC melting curves. These results were in agreement with previous studies on the melting profiles of other seed oils obtained from various extraction methods, such as cold pressing, solvent, supercritical fluid extraction CO_2 , ultrasound assisted, and aqueous enzymatic extraction (Abdulkarim et al., 2005; Albakry et al., 2023; Samaram et al., 2014).

In the case of peak height, there was a highest variability in the first peak height ($hm1$) of blackcurrant seed oil from n-hexane extraction, which is 0.16 W/g with 40% of relative standard deviation (RSD). In contrast, low RSD values were observed in cold-pressed ($hm1 = 0.18\text{ W/g}$, 13% RSD) and supercritical oils ($hm1 = 0.23\text{ W/g}$, 13% RSD) as presented in Table 1. Among all extraction methods, highest $hm1$ were observed in cold-pressed oils, particularly in cold-pressed raspberry- (0.6 W/g) and strawberry- (0.43 W/g), excluding blackcurrant seed oils. Cold-pressed blackcurrant oils possessed a lower $hm1$ than supercritical extraction, but slightly higher than n-hexane extraction. This observation might be explained by the previously-mentioned pectin content in blackcurrant oil, lowering the triacylglycerols content in the oils.

Despite having a minimal effect on the shape of DSC melting curves, a slight shift towards a higher temperature was also observed for the second endothermic peak (T_{m2}). Oils obtained using cold-pressing techniques tend to have higher temperatures in T_{m2} than for hexane and supercritical extraction. In contrast, such differences were not observed in the first endothermic peak (T_{m1}) regardless of the extraction method. Peak shifting in oils from different extraction methods was also reported in another study. For instance, in the melting profile of black cumin seed oil, obtained by supercritical fluid extraction, Albakry et al., (2023) observed a shift of $3\text{ }^{\circ}\text{C}$ in the main peak towards higher temperatures compared to oils obtained by cold pressing and solvent extraction.

In DSC melting curves of berry seed oils, the first endothermic peak at T_{m1} represents low-melting triacylglycerol (TAG) fraction, while second endothermic peak at T_{m2} is associated with high-melting TAG (Piasecka et al., 2021). The TAG distribution is indicated by the correlation of T_{m1} with $c18:1$ ($r=0.63$) and $c18:2$ ($r=-0.86$), as well as T_{m2} with $c16:0$ ($r=-0.51$), $c18:2$ ($r=-0.49$), $c18:3\text{ n-3}$ ($r=0.65$), and $c18:3\text{ n6}$ ($r=-0.76$), as shown in Fig. 4A. These correlations are in agreement with the recent findings about lipid markers of blackcurrant,

raspberry, and strawberry seed oils accountable for berry types differentiation (Kozub-Pędrak et al., 2025). The authors reported the distribution of the aforementioned fatty acids in TAG markers of each berry oils, such as TAG 56:6 (c18:2, c18:3, c20:1) for blackcurrant, TAG 38:4 (c18:2, c18:2, c2:0) for raspberry, and TAG 52:7 (c16:1, c18:3, c18:3) for strawberry seed oils. Thermal history of a sample may induce the TAG rearrangement and lead to polymorphism as observed in DSC melting range and peak shapes (Micić et al., 2015). The first endothermic peak (area under Tm1) contains metastable polymorph, it melts upon heating and recrystallises into a stable polymorph at the second endothermic peak (area under Tm2). The presence of an exothermic peak for raspberry seed oil (Tm3) is evidence of polymorphic transitions occurring during melting. Consequently, differences in peak height and slight shifting appeared in oil from different extractions.

Considering the similarity of fatty acid composition across extraction methods, the shifting of Tm2 in cold-pressed samples can also be partially explained by the correlation with carotenoid ($r=-0.42$) and chlorophyll ($r=-0.71$). Both bioactive compounds were retained at higher concentrations through cold-pressing compared to oils obtained by n-hexane and supercritical CO₂ extractions, where the solvent use and relatively high processing parameters (i.e. temperature, pressure) contributed to reduced chlorophyll and carotenoid contents. Aside from bioactive compounds, the presence of minor components such as free fatty acids, partial glycerides, and oxidation products were known to influence endothermic peak shifting to lower temperatures (Tan and Man, 2000). This was confirmed by a recent study about the removal of minor components that influenced fat crystal network of edible oil, resulting in the polymorphic changes marked by differences in DSC peaks height and peaks temperature shifting (Chai et al., 2018).

To visualise the data trend based on ‘berry oil types’ and ‘extraction methods’, the entire DSC melting profiles constituting the data matrix of 54x464 variables of normalised heat flow were taken for PCA. The PCA score plot shown in Fig 3D, with PC1 and PC2 explaining 72.10% and 18.64% variance, demonstrated clear separation between three berry oil types based on the full melting curves. However, there was no clear separation between oils in terms of extraction methods. This result emphasised the limitations of DSC in authenticity study, as it is sensitive in differentiating the general peak shape but less sensitive in detecting the slight shift caused by different extraction techniques. This observation is in agreement with the study by Oomah and Sitter on flaxseed oil extracted by different methods (Oomah and Sitter, 2009).

3.2.2 FTIR spectra as affected by oil extraction methods

The FTIR spectra of berry seed oils were recorded from 500 to 4000 cm^{-1} (Fig 3E). Different berry types and extraction techniques influenced the absorption intensity, as observed in various FTIR peaks. When applied to the whole spectra (54x3501 variables), PCA (Fig 3H) revealed a clear separation between extraction techniques (PC1=67.31%, PC2=17.18% variance). This result is in agreement with a previously reported study on the FTIR spectra of black cumin seed oil from cold pressing, solvent, and supercritical CO_2 extractions (Albakry et al., 2023). The authors reported that the black cumin oils from different extractions can be distinguished at the range 2800 – 3000 cm^{-1} and 1000 – 1500 cm^{-1} . The most important data points related to extraction groups were obtained from PC1 (wavenumber 2931, 1726) and PC2 (wavenumber 1408, 1046) (Fig 3F, 3G). These data points corresponded to specific functional groups commonly found in edible oils, such as -C-H(- CH_2) in Wn 2931, -C=O (acid) in Wn 1726, =C-H(cis-) in Wn 1408, and -C-O in Wn 1046 (Jamwal et al., 2021).

The correlation of these FTIR wavenumbers with physicochemical analysis is determined in Fig 4A. PCA biplot of FTIR data (Fig 4B) illustrates the relationship between sample from different extractions and important wavenumbers for differentiation. Wavenumber (Wn) 1726 is closely related to supercritical CO_2 and some cold-pressed samples. These relationships were marked with strong correlation in DPPH ($r=0.51$), γ -tocopherol ($r=0.49$), acid value ($r=0.42$), chlorophyll ($r=-0.39$), chroma ($r=-0.38$), and fatty acids content i.e. c18:1 ($r=-0.39$); c18:2 ($r=0.46$); c18:3n-3 ($r=0.37$). Both extractions provide milder processing condition, resulting in better preservation of heat-sensitive antioxidants like γ -tocopherol. As Wn 1726 corresponds to the stretching of -C=O (acid) bonds, band intensities directly indicate the prominent FFA presence in supercritical CO_2 oils (Fig 2A).

The rest of cold-pressed oils spread closer towards Wn 2931 and Wn 1408. While Wn 2931 mostly represents visual characteristics as chroma ($r=0.34$) and chlorophyll ($r=-0.28$), Wn 1408 coverage are similar to Wn 1726 that correlates to antioxidant activity (r DPPH =0.54), γ -tocopherol content ($r=0.48$), acid value ($r=0.47$), chlorophyll ($r=-0.42$), chroma ($r=-0.44$), and fatty acids: c18:1 ($r=-0.43$); c18:2 ($r=0.49$); c18:3n-3 ($r=0.39$). Although correlation with fatty acids was found, it is still weaker than those found in DSC melting peaks. The correlations with two wavenumbers not only indicate the overall similarity in antioxidant properties of cold-pressed oils and supercritical CO_2 , but also highlighted the most distinctive visual features of cold-pressed oils. Contrary to previous clusters, Wn 1046 was mostly correlated with quality parameters i.e. PV ($r=-0.62$); p-AnV ($r=-0.39$); AV ($r=0.43$); K_{268} ($r=-0.41$), indicating higher quality deterioration occurred in hexane-extracted oils.

Nevertheless, similar correlations with chroma ($r = 0.38$ to 0.51) and chlorophyll ($r = -0.41$ to -0.39) were observed in wavenumber 1046, 1408, and 1726. Excluding the fatty acids composition and K_{268} concentration, each extraction method produced oils with distinctive physicochemical properties, as suggested by FTIR spectra and its combination of quality parameters as discussed earlier in Chapter 3.1.

In contrast to DSC melting profiles, berry oil types could not be recognised solely using FTIR spectra and PCA. This limitation was not improved even though a different selection procedure was applied. This result emphasised the advantages of combining DSC and FTIR data to achieve the aim of the current work, noting that DSC data were highly correlated with parameters related with antioxidants, oxidative stability, and fatty acids. FTIR data can complement the data coverage with its correlation on quality parameters and bioactive components. The characteristics of both datasets opened up the possibility to fuse both datasets into one and utilise this for more advanced purposes.

3.3 Rapid classification of berry types and extraction methods using data fusion and SIMCA

Low-level data fusion was carried out using a pre-processed version of both DSC and FTIR datasets - this new dataset will be further referred to as “DSC-FTIR”. Three-dimensional PCA (Fig 5) revealed a clear trend dividing oils by ‘berry oil types and extraction methods’. In both strawberry and raspberry clusters, cold-pressed and supercritical oils were located close to the each other. This resembles the FTIR spectra trend at Wn 1408 and 1726, representing antioxidant properties. In contrast, cold-pressed and hexane-extracted blackcurrant oils clustered together, reflecting their similar dark visual as indicated by L^* and chroma values. Integration of both datasets provides horizontal distance in score plots to separate oil based on berry types and vertical separation for extraction methods.

This result indicates the potential capability of the newly fused dataset to distribute the group properly without previously establishing assumptions. Hence, the prospective classification model was more likely to be built based on the similarities among the samples rather than focusing on the differences with other groups’ characteristics, e.g. PLS-DA. With respect to these attributes, a SIMCA algorithm was employed to build the models. The SIMCA performance from DSC-FTIR was assessed according to its capabilities in classifying berry oil types, extraction methods, and both grouping categories at the same time against the plain DSC and FTIR data (Table 2). The models’ performance was tested using internal cross-validation (k-fold venetian blinds) and external validation. Values of performance metrics (sensitivity, specificity, accuracy, F1 score) were calculated from the accumulation of predictions across

iterations. The uncertainty of these values were estimated using 95% Clopper-Pearson confidence intervals, which provide the lower and upper bounds of the true classification performance.

In the classification of ‘berry oil types’, DSC calibration model exhibited the highest performance against DSC-FTIR and FTIR calibration models. It is marked by the highest sensitivity (98%), specificity (100%), accuracy (99%), and the F1 score (99%). This characteristic is consistent with the trend observed in PCA score plot for DSC data (Fig 3D). Despite the superior calibration performance, DSC model was outperformed by DSC-FTIR during external validation. DSC-FTIR validation model exhibited the highest sensitivity (63%), specificity (82%), accuracy (72%), F1 score (69%), as well as relatively high scores for confidence intervals (CIs) in all metrics. In contrast, plain DSC and FTIR models performed similarly in validation subset as indicated by their respective sensitivity (44%, 48%), specificity (72%, 74%), accuracy (58%, 61%), F1 scores (52%, 55%), and similar CI range in all metrics. It should be noted that, the overall CIs for validation performance metrics were wider than those for calibration. It indicates a higher uncertainty of model performance and implies the need of larger validation set.

During the classification of ‘extraction methods’, FTIR calibration model exhibited the highest accuracy (96%), specificity (98%), accuracy (97%), and F1 score (97%) against DSC-FTIR and plain DSC models. Generally, the plain FTIR model performed better than DSC model in classifying extraction methods. These results corresponded to its properties, as discussed in Chapter 3.2.2. In validation subset, both DSC-FTIR and FTIR models performed similarly, as indicated by the same scores in all performance metrics. Lowest performance to classify extraction method was found in DSC validation model. The performance comparison between DSC-FTIR, DSC, and FTIR models for classifying ‘berry oil types’ and ‘extraction methods’ verified that data fusion provides greater insights than individual analytical techniques.

When calibration models were subjected to classify ‘berry oil types and extraction methods’ at the same time, lower F1 scores and accuracy were observed in all models compared the values from single classification category. Despite the reduced performance, DSC-FTIR models performed better in both calibration and validation subsets compared to plain DSC and FTIR models as indicated by higher values in sensitivity (66%, 60%), accuracy (81%, 77%) and F1 scores (78%, 73%). It is followed by FTIR calibration and validation models that performed slightly better than DSC in specificity (97%, 97%), accuracy (68%, 67%), F1 scores (52%, 52%), and higher CI lower bounds. Lowest performance to classify both categories at

the same time was exhibited by DSC calibration and validation models, marked by their respective performance metrics in sensitivity (38%, 35%), specificity (92%, 92%), accuracy (65%, 64%), and F1 scores (52%, 49%).

Table 3 provides a detailed summary of DSC-FTIR calibration model's performance to classify the 9 classes. To observe per-class performance and correct classification, F1 score and accuracy were used as the main metrics in comparison table. The DSC-FTIR model exhibited the highest accuracy (68% to 100%) in most classes. Additionally, FTIR model exhibited the highest accuracy to classify ST-P (100%) and RB-S (80%) classes. DSC models exhibited the lowest accuracy scores in 5 classes (50% in BC-P, 53% in BC-S, 50% in RB-S, 50% in ST-P, 50% in ST-S). These results are consistent with F1 score of each models across 9 classes. DSC-FTIR exhibited comparatively more balanced F1 scores (48% - 100%), followed by FTIR (6% - 100%), then DSC (0% - 96%). These results highlight that certain classes were more challenging to classify than others. For instance, the plain DSC model faced difficulties to classify strawberry oils from different extractions (F1 scores = ST-E 50%, ST-P 0%, ST-S 0%). Similarly, the plain FTIR model showed poor performance in separating hexane-extracted blackcurrant and strawberry oils (F1 scores = BC-E 28%, ST-E 18%). These limitations were mitigated by combining both DSC and FTIR data through data fusion, resulting in better performance to classify both categories at one run.

Summing up, the SIMCA model built based on DSC-FTIR data fusion registered excellent performance in the rapid classification of berry oil types and extraction methods. It is potentially feasible to use this approach during oil-receiving screening to avoid false claims made by fraudsters. Additionally, this chapter highlights the advantages and limitations of using raw analytical instruments data during rapid screening. It also introduces the low level of data fusion techniques for DSC and FTIR, in order to draw more meaningful insights during food safety and authenticity screening.

4. Conclusions

Different extraction methods of berry seed oils affected most of physicochemical parameters, except fatty acid composition. Each extraction method resulted in distinguishable physicochemical characteristics. Although deciding the most appropriate methods is highly dependent on producer's decision-making, the decision process should start from physicochemical properties, food safety, and economic aspects, respectively. Extraction methods affected DSC thermal profiles by a slight shifting of one endothermic peak. The shifting occurred consistently across all berry oil types. In FTIR, the influence of extraction

methods was more prominent and the grouping trend was recognisable in PCA. Both analytical techniques were highly correlated with conventional analysis. DSC parameters correlated strongly with antioxidant activity, antioxidant content, oxidative stability, and fatty acid, while FTIR registered strong correlations with quality parameters and bioactive components. By using low-level data fusion and optimised pre-processing, the DSC-FTIR dataset demonstrates the potential in enhancing the classification of designated groups compared to plain instrumental data. This establishes proof-of-concept for combining spectral data with thermal analysis to achieve robust classification during oil analysis and food safety screening. Despite the promising results, this performance can be further improved by using a larger number of samples during external validation and additional oil types with greater sample diversity (i.e. botanical origin, geographical origin, harvest years, etc).

Ethical statements

This study did not include any human subjects and animal experiments

CRediT authorship contribution statement

Yolanda Victoria Rajagukguk: Conceptualisation, Data curation, Visualisation, Formal analysis, Methodology, Investigation, Funding acquisition, Writing – original draft. **Sylwia Ryszczyńska:** Investigation. **Anna Grygier:** Investigation. **Aleksander Siger:** Investigation. **Agnieszka Waśkiewicz:** Investigation. **Jolanta Tomaszewska-Gras:** Conceptualisation, Supervision, Resources, Methodology, Writing – review & editing.

Data availability

Data will be made available on request.

Declaration of competing interests

The authors declare that there is no competing interest.

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Table 1

Parameters of peak temperature and peak height of DSC melting curves of strawberry-, raspberry- and blackcurrant seed oils obtained by cold pressing, n-hexane and supercritical CO₂ extraction

Extraction method		Peak temperature (°C)			Peak height (W/g)		
		Tm1 (endo)	Tm2 (endo)	Tm3 (exo)	hm1 (endo)	hm2 (endo)	hm3 (exo)
Cold pressing	Strawberry	-33.59 ± 0.28	-17.42 ± 0.32	-	0.43 ± 0.03	0.01 ± 0.00	-
	Raspberry	-38.30 ± 1.08	-20.57 ± 2.05	-30.67 ± 1.93	0.60 ± 0.08	0.62 ± 0.01	-0.12 ± 0.07
	Blackcurrant	-36.86 ± 0.29	-25.62 ± 0.59	-	0.18 ± 0.02	0.01 ± 0.00	-
n-Hexane	Strawberry	-33.82 ± 0.23	-20.82 ± 0.47	-	0.37 ± 0.01	0.04 ± 0.02	-
	Raspberry	-37.55 ± 0.48	-24.99 ± 0.37	-30.06 ± 1.01	0.55 ± 0.07	0.12 ± 0.08	-0.01 ± 0.00
	Blackcurrant	-36.55 ± 0.34	-27.88 ± 0.62	-	0.16 ± 0.06	0.03 ± 0.00	-
Supercritical CO ₂	Strawberry	-34.28 ± 1.26	-18.46 ± 0.89	-	0.34 ± 0.03	0.07 ± 0.02	-
	Raspberry	-37.84 ± 0.63	-24.15 ± 0.88	-30.26 ± 0.47	0.59 ± 0.03	0.26 ± 0.11	-0.02 ± 0.01
	Blackcurrant	-37.13 ± 0.30	-26.02 ± 0.43	-	0.23 ± 0.03	0.02 ± 0.01	-

Table 2

SIMCA models information and performance comparison between DSC-FTIR, DSC, and FTIR datasets to classify ‘berry oil types’, ‘extraction methods’, and both categories at one run.

SIMCA (most probable, 95% Clopper-Pearson CI)	Dataset	Berry oil types		Extraction methods		Berry oil types & extraction	
		Calibration	Validation	Calibration	Validation	Calibration	Validation
Sensitivity (%)	DSC-FTIR	97 (62 - 100)	63 (27 - 91)	89 (52 - 100)	54 (20 - 85)	66 (52 - 79)	60 (46 - 72)
	DSC	98 (64 - 100)	44 (14 - 79)	77 (39 - 97)	43 (12 - 77)	38 (25 - 52)	35 (23 - 49)
	FTIR	87 (50 - 99)	48 (16 - 81)	96 (61 - 100)	54 (20 - 85)	36 (23 - 50)	35 (23 - 49)
Specificity (%)	DSC-FTIR	99 (64 - 100)	82 (44 - 98)	94 (59 - 100)	77 (39 - 97)	96 (87 - 99)	96 (86 - 99)
	DSC	100 (66 - 100)	72 (35 - 95)	89 (51 - 100)	71 (34 - 95)	92 (82 - 98)	92 (81 - 98)
	FTIR	94 (57 - 100)	74 (36 - 96)	98 (64 - 100)	77 (39 - 97)	97 (88 - 100)	97 (88 - 100)
Accuracy (%)	DSC-FTIR	98 (78 - 100)	72 (47 - 90)	92 (69 - 99)	65 (40 - 86)	81 (73 - 88)	77 (68 - 84)
	DSC	99 (80 - 100)	58 (33 - 81)	83 (58 - 96)	57 (32 - 80)	65 (55 - 74)	64 (54 - 73)
	FTIR	90 (67 - 99)	61 (36 - 83)	97 (77 - 100)	65 (40 - 86)	68 (58 - 76)	67 (58 - 76)
F1 score (%)	DSC-FTIR	98	69	91	61	78	73
	DSC	99	52	82	50	52	49
	FTIR	90	55	97	61	52	52
Sample size		n=36 (12 per class)	n=18 (6 per class)	n=36 (12 per class)	n=18 (6 per class)	n=45 (5 per class)	n=9 (1 per class)
Class		3 (BC, RB, ST)		3 (E, P, S)		9 (BC-E, BC-P, BC-S, RB-E, RB-P, RB-S, ST-E, ST-P, ST-S)	
CV (venetian blinds)		12-fold		12-fold		5-fold	
Iterations		3		3		6	

CV: cross validation, CI: confidence interval, BC: blackcurrant, RB: raspberry, ST: strawberry, E: n-hexane, P: cold pressing, S: supercritical CO₂. Values are calculated from pooled predictions across iterations with 95% Clopper-Pearson CI (lower to upper bound).

Table 3

Percentage of accuracy in SIMCA calibration models (most probable rule) for rapid classification of ‘berry oil types and extraction methods’

Class		Accuracy (%), 95% CI			F1 score (%)		
		DSC-FTIR	DSC	FTIR	DSC-FTIR	DSC	FTIR
BC	E	89 (59 - 99)	88 (57 - 99)	56 (26 - 83)	89	90	28
	P	68 (36 - 91)	50 (21 - 79)	55 (25 - 83)	54	0	18
	S	100 (74 - 100)	53 (24 - 81)	58 (27 - 85)	100	13	28
RB	E	100 (74 - 100)	96 (67 - 100)	83 (52 - 98)	100	96	80
	P	81 (50 - 97)	81 (49 - 97)	52 (22 - 80)	83	84	6
	S	70 (36 - 93)	50 (21 - 79)	80 (48 - 97)	50	0	75
ST	E	68 (34 - 92)	67 (35 - 90)	55 (25 - 83)	48	50	18
	P	82 (50 - 97)	50 (21 - 79)	100 (74 - 100)	78	0	100
	S	70 (36 - 92)	50 (21 - 79)	68 (36 - 91)	51	0	54

CI: confidence interval, BC: blackcurrant, RB: raspberry, ST: strawberry, E: n-hexane, P: cold pressing, S: supercritical CO₂. Values are calculated from pooled predictions across iterations with 95% Clopper-Pearson confidence interval (lower to upper bound).

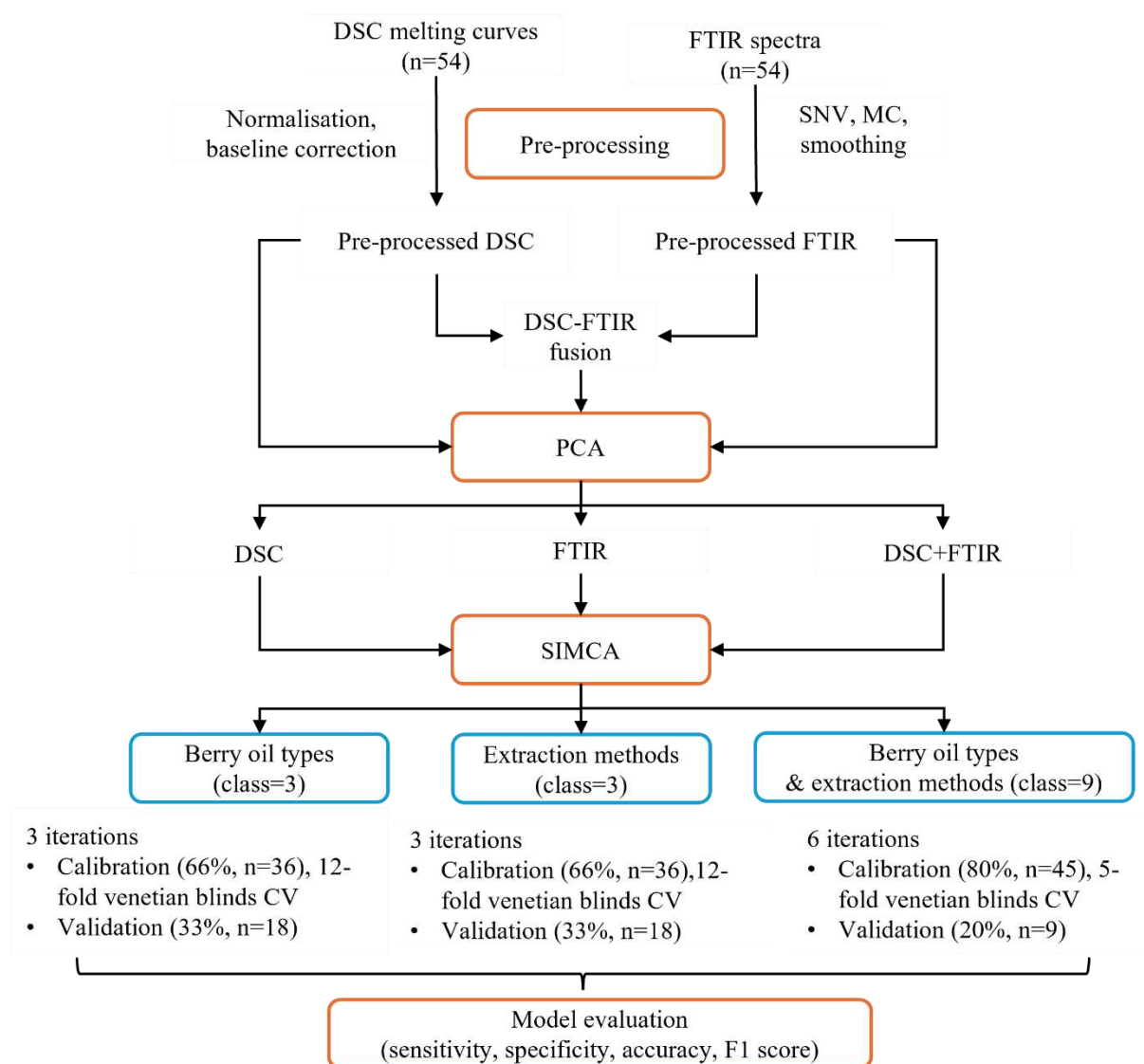


Fig. 1. Chemometrics workflow from pre-processing to SIMCA model evaluation for all datasets.

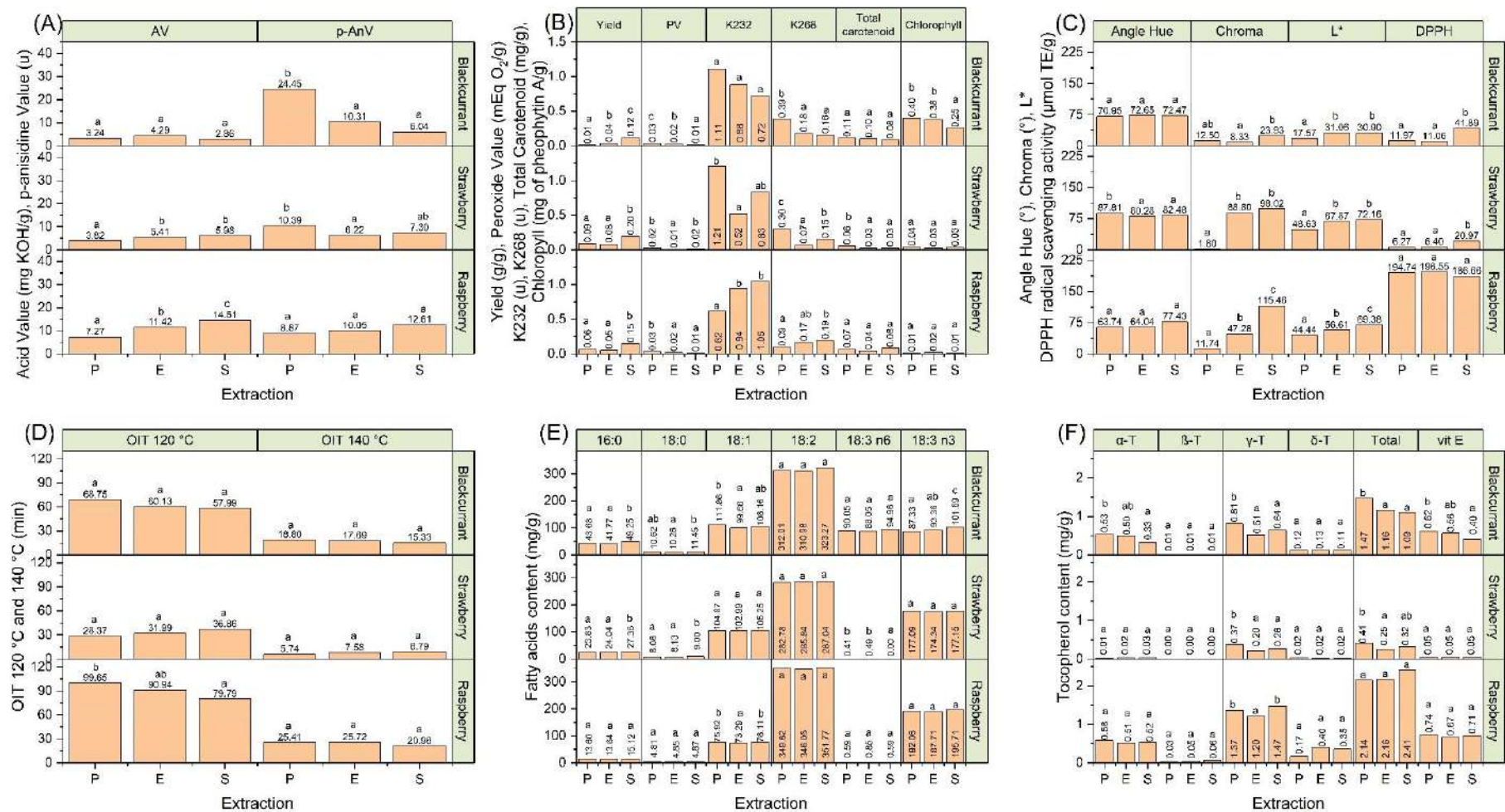


Fig. 2. Physicochemical characteristics of berry seed oils obtained from different extraction methods. Bars marked with a, b, c indicate statistically significant values observed within the same oil from different extraction methods ($p < 0.05$). P: cold pressing, E: n-hexane, S: supercritical CO₂ extraction.

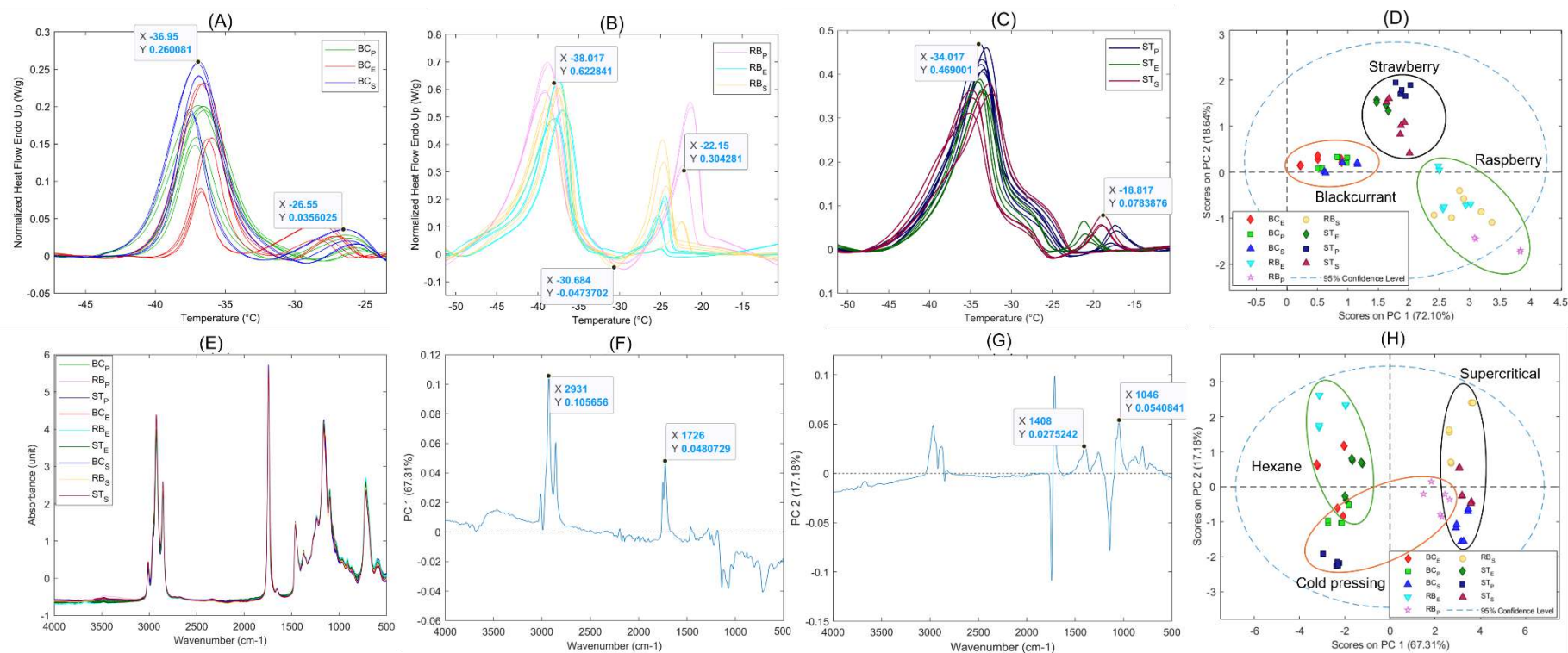


Fig. 3. DSC melting profiles of berry seed oils: (A) blackcurrant, (B) raspberry, (C) strawberry. PCA scores plot from DSC data (D). FTIR spectra of oils from different extraction methods (E). PCA loadings for PC1 and PC2 (F,G) and PCA scores plot from FTIR data (H).

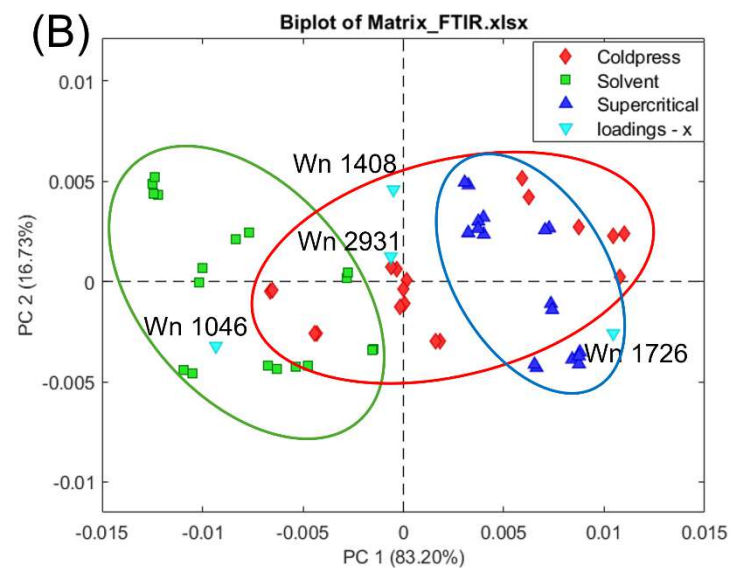
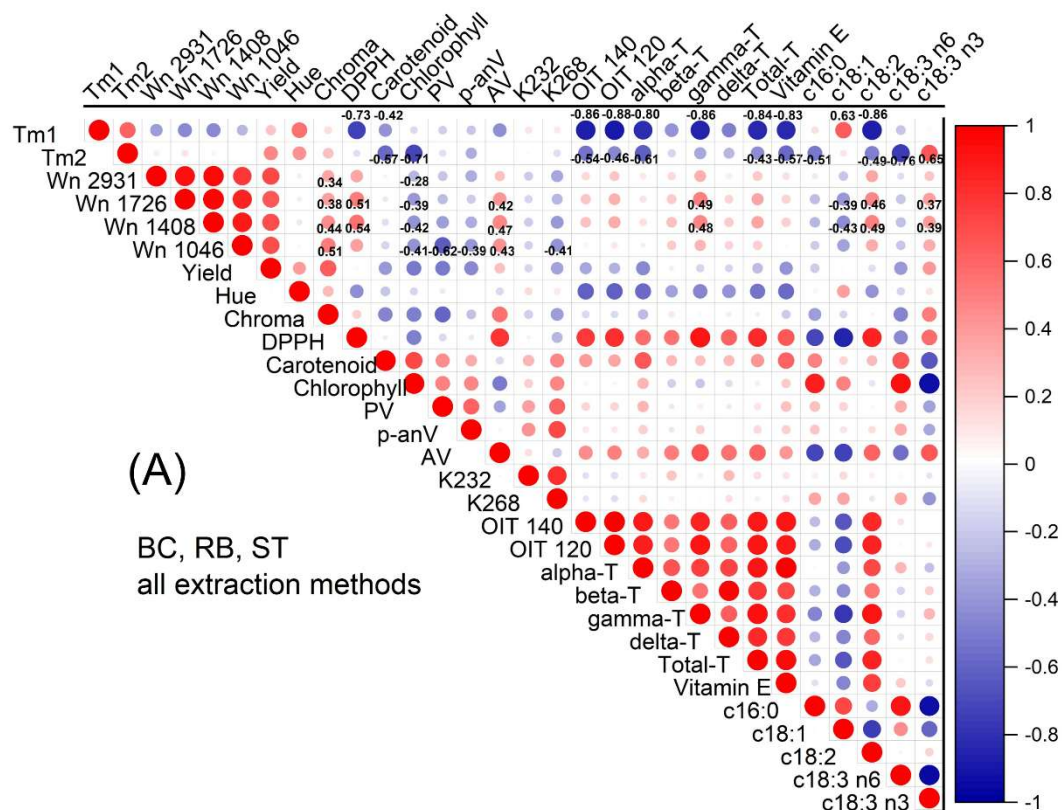


Fig. 4. Pearson's correlation of physicochemical, thermal, and spectroscopic properties of berry seed oils obtained by different extraction methods (A). PCA biplot of FTIR data, illustrating the relationship between oil from different extraction methods with specific wavenumbers (Wn) (B).

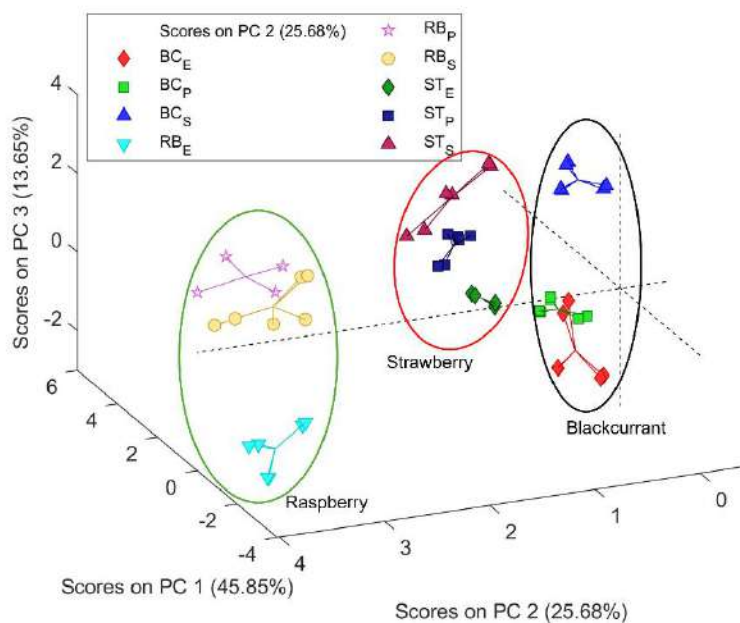


Fig. 5. PCA scores plot from fused DSC-FTIR dataset, illustrating the separation of samples into nine classes ‘berry oil type & extraction method’.

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