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Review Report on the PhD thesis

Author: **Mgr Mahbuba Islam**

Title: **The application of Differential Scanning Calorimetry (DSC) for the quality assessment of selected edible oils**

Supervisor: **Dr hab. Jolanta Tomaszewska-Gras, prof. UPP**

Academic supervisor: **Dr inż. Anna Kaczmarek**

1. Assessment of choice and importance of the undertaken research areas

Recently, cold-pressed oils have received increased attention due to their health-beneficial impact. The growing popularity of cold-pressed oils among consumers is related to less processed products containing higher amounts of bioactive substances and minerals that are not being removed during refining processes. However, one of the most critical factors influencing the quality and nutritional value of cold-pressed oils is the oxidation process of unsaturated fatty acids as well as the degradation and volatilisation of bioactive compounds. Primary (peroxides) and secondary (aldehydes, ketones, and hydrocarbons) oxidation products are associated with rancid oils and damage to health, lowering consumer acceptance. It is known that cold-pressed oils have a higher initial oxidation level because they are made only by mechanical pressing of seeds, and thus, oxidation products are not removed. Therefore, the oxidative stability of cold-pressed oils depends not only on the composition of fatty acids but also on the contents of primary and secondary oxidation products, antioxidants, prooxidants, metals and other contaminants, as well as processing and storage conditions that can accelerate or inhibit the oxidation processes.

Although there are many classical and instrumental analytical methods for the determination of oil oxidation products in the literature, they have some drawbacks, such as laborious chemical processes, use of toxic solvents and long analysis times.

Among well-known methods that measure the extent of oil oxidation, such as the Rancimat test, determination of peroxide (PV) and anisidine (AnV) values, spectrophotometric and chromatographic analyses of oxidation products, differential scanning calorimetry (DSC) is widely used as an analytical, diagnostic and research tool from which relevant information, such as onset temperature of oxidation, height, shape and position of peaks are obtained and used for subsequent kinetic calculations. On the other hand, fatty acid and triacylglycerol composition inherent to each fat type results in a unique melting or crystallization pattern. Therefore, DSC can be a prominent analytical technique to evaluate the adulteration of vegetable oils and their quality. The assessment of oil and fat authenticity predominantly focuses on studying crystallization and melting profiles. The adulteration of cold-pressed oils not only seriously undermines consumers' interests but also poses potential health risks. Consequently, rapid and accurate recognition of oil kind, its authenticity, and oxidative deterioration have become increasingly important in recent years. Moreover, chemometrics can successfully process large and diverse thermal data to characterise the quality of cold-pressed oils.

For this reason, the PhD thesis of Mahbuba Islam is devoted to evaluating the use of DSC coupled with chemometric tools such as principal component analysis (PCA), orthogonal partial least squares-discriminant analysis (OPLS-DA), linear discriminant analysis (LDA), multiple linear regression (MLR), support vector machine (SVM), artificial neural networks (ANN), multivariate adaptive regression splines (MARS), and partial least squares (PLS) to (1) detect the oxidative stability for fresh and stored three cold-pressed oils (flaxseed oil, camelina oil and hempseed oil), (2) verify the authenticity of these oils and to (3) identify and quantify the presence of adulterants (refined rapeseed oil) in cold-pressed flaxseed oil. Additionally, amounts of primary and secondary oxidation products and free fatty acids, as well as color and antioxidant capacity tests for the oxidative and hydrolytic state of the studied cold-pressed oils, were also assessed using official and well-known analytical methods.

Thus, this PhD thesis emphasizes that the DSC technique coupled with chemometric analysis can be used to determine melting profile characteristics and crystallization, the oil oxidation stability, and the detection, discrimination, and quantification of cold-pressed oils adulterated with cheaper refined vegetable oils.

2. Formal assessment of the dissertation

The reviewed PhD thesis of Mahbuba Islam is based on the coherent collection of six scientific articles published in 2021-2023 in international journals indexed in Journal Citation Reports (JCR). The total Impact Factor of these six publications is high and equal to IF = 25.5 (total MEiN = 700), which gives the average IF value = 4.25 (average MEiN = 116.7) per article. Articles constituting dissertation were published in journals from quartile 1 (Q1) – two articles (A3 and A5), quartile 1/2 (Q1/Q2) – one article (A2), quartile Q2 – one article (A1), and quartile Q3 – one article (A4), respectively. The individual output of PhD student was declared in the range of 45 - 51%, which indicates that her participation in developing the research plan (conceptualization), conducting studies (methodology, formal analysis, investigation), results interpretation (data curation, visualization) and writing research articles was significant. However, the PhD student is the first author in five articles, but in none of them, she is not the corresponding author.

The articles' dissertation format forms a cohesive body of work concerning themes expressed clearly in the attached commentary consisting of five chapters. The scientific quality of these articles and their excellent scientific level were confirmed by the experts selected for revision processes.

The doctoral thesis has 164 pages (104 pages contain articles reprints) and is written in English. It consists of the following structure: Title page, Dedication, Acknowledgement, Summary in English and Polish, Table of contents, List of publications, Introduction (chapter 1), Materials and methods (chapter 2), Research goals and hypotheses (chapter 3), Results (chapter 4), which is a description of the content of six publications, Conclusions (chapter 5), List of Figures, References (82 items) arranged alphabetically, Keywords (37 items), and reprints of six articles constituting the dissertation with statements about the contribution of authors after each article.

A separate document presents the scientific achievements of Mahbuba Islam, which includes 7 additional publications (beyond the dissertation themes) in journals from the JCR list (total IF = 24.0, total MEiN = 760), 9 presentations (3 oral) during international (6) and national (3) conferences, and participation in training programs (4). Therefore, the effects of her research were included in 13 publications (in 8 of them, she was the first author) in journals listed in JCR (total IF = 49.5, total MEiN = 1460) and were cited in a total of 38 times according to Web of Science database (10 November 2023).

The research constituting this PhD thesis and total scientific achievements of Mahbuba Islam could be carried out thanks to financial support and cooperation in three research projects: (1) "Identification of lipidomic biomarkers for the authenticity of edible oils supported by DSC profiling and chemometrics", Project grant number: 2018/31/B/NZ9/02762, (2) National Center for Research and Development- (NCBR) Project details: Operational Program Knowledge Education Development (PO WER), and (3) "Innovative technologies of separation and use of poultry blood components for animal nutrition", Project No. 52/2021/U.

Moreover, she was awarded the Erasmus plus scholarship and completed three months of traineeship at the Federal Institute for Food and Nutrition Research - Max Rubner Institute, Germany, under the supervision of Professor Dr. Bertrand Matthäus. She also obtained 1st Place in Poster Session IXth International Session of Young Scientific Staff "Food nowadays local or global? traditional or innovative?", Poznań 2022 from the Scientific Commission Award.

3. Substantive assessment of the dissertation

Commentary on a monothematic cycle of publications constituting a doctoral dissertation is divided into five sections, which are typical for scientific articles.

The title of the reviewed dissertation was formulated correctly in relation to the included publications.

The first section, "Introduction", was prepared based on an explicit and robust literature review. This chapter provides basic information about the production of cold-pressed oils, their quality and health, as well as global production with economic data. In addition, the fatty acid compositions and bioactive compounds in three cold-pressed oils (flaxseed oil,

camelina oil, and hempseed oil) and their effect on health benefits and nutritional properties were compared and described. It was noted that the present unsaturated acids affect the oxidative stability of cold-pressed oils. Therefore, analytical methods for the determination of the oxidation status of edible oils were mentioned, limited to the official procedures used by the fat industry. **Although attention was paid to the disadvantages of these standard analytical tests, other well-known assays for the oxidative stability of edible oils assessment were not described.** However, the possibility of DSC as a prominent analytical technique for the evaluation of oxidative degradation, fraud detection (mainly cheaper refined vegetable oils), quality assessment, traceability, and polymorphism investigation of edible oils and fats was clearly highlighted. A separate paragraph of the "Introduction" section reported the importance of several chemometric tools coupled with different instrumental methods (thermal, spectroscopic and chromatographic) for the characterization, authentication and quality control of virgin olive oils and cold-pressed oils. Taking into account consumer health protection, extraordinary quality, economic aspects, and the high commercial value of cold-pressed oils, the DSC method combined with chemometric tools was proposed for the determination of the oxidative stability and authenticity of three cold-pressed oils. Therefore, the research plan mainly focusing on (1) a comprehensive evaluation of oxidative stability for fresh and stored oils, (2) the application of DSC profiling and advanced chemometric methods for verification of the authenticity of flaxseed oil, camelina oil, and hempseed oil during shelf life, (3) identification and quantification of refined rapeseed oil in cold-pressed flaxseed oil, was presented in a diagram. This graphical presentation ensures clear and understandable aims and objectives of the reviewed dissertation.

In the second "Materials and methods" section, (1) various cultivars of flax, camelina, and hemp seeds, (2) conditions of cold-pressing oil samples from them and storage, (3) commercially available refined oils used for adulteration, and (4) the analytical methods (determination of fatty acid composition, color, characteristic values expressed oxidative status, radical scavenging activity by 2,2-difenylo-1-pikrylohydrazyl (DPPH) assay, oxidative stability and melting and crystallization profiles by the proposed DSC technique) applied to achieve the assumed PhD thesis goals were listed in detail. This section ends with statistical and chemometric tools to derive relevant information from large and diverse datasets, such

as thermal data. It is important to emphasize that applying proposed adequate multivariate linear and non-linear methods for analyzing complex datasets, classification, pattern recognition, optimization, and prediction can solve demanding analytical problems in the quality and safety of cold-pressed oils.

The research methodology and the analytical procedures descriptions are presented in a detailed and sufficient way that allows for their repetition. Additionally, the proposed instrumental methods are among the modern ones eagerly used by researchers.

In my opinion, this comprehensive collection of the tested materials and analytical methods in the second section could be omitted because they were presented in the attached articles (materials - in each publication, fatty acid composition - in A1, A2, A3; color measurement - in A1, A2; chemical determination of oxidative stability - in A1, A2, A3, A6; radical scavenging activity by DPPH - in A2; determination of oxidative stability by DSC - in A1, A2, A6; DSC melting and crystallization analysis - in A1, A3, A4, A5). It might be enough to illustrate the analytical methods in a diagram, similar to Figures 1 and 2, showing the research plan and oils procured for the experiment, respectively.

In the third "Research goals and hypotheses" section, the PhD candidate clearly and precisely formulated one main goal, which was achieved by implementing five specific goals. Moreover, the presented hypotheses are based on previous observations, research and theories and are presented clearly and understandably. They are closely related to the title of the dissertation and define the scope of subsequent research tasks, which facilitates the assessment of their implementation. **The most significant deficiency is the lack of a formulated main hypothesis.**

The fourth "Results" section was divided into three subsections containing the results of (1) oxidative stability of selected fresh and stored cold-pressed oils based on isothermal and non-isothermal measurements by DSC, (2) authenticity and stability of cold-pressed and refined oils through the DSC phase transition profiles, and (3) detection of adulterants (refined rapeseed oil) in cold-pressed flaxseed oil using DSC melting profiles coupled with chemometric methods. The obtained results were presented skillfully and comprehensively based on a coherent collection of 6 publications.

The obtained results of oxidation parameters (also presented in A1 and A2) showed that the type of oil (mainly fatty acid profile of oilseed cultivar) and heating temperature and

rate affected the shapes, width and height of the peaks of DSC isothermal and non-isothermal oxidation curves, as well as DSC crystallization and melting curves of oils cold-pressed from five varieties of flaxseeds and three varieties of camelina seeds. Thus, differences in oxidation parameters such as oxidation induction time, oxidation end time, length of oxidation, and rate of oxidation as well as onset and end temperatures calculated from DSC isothermal and non-isothermal curves, respectively, for cold-pressed flaxseed and camelina oils provide an accurate assessment and comparison of their oxidative stability. In addition, high determination coefficients (R^2 = 0.90 - 0.99) for relationships between oxidation parameters from DSC isothermal and non-isothermal curves from cold-pressed camelina oils confirm the adequacy and fitness of generated equations for the prediction of the oxidation process of these oils.

Interestingly, the calculated kinetic parameters (activation energy, half-life times, and oxidation rate constants) from the generated DSC curves allow the ranking of camelina oils pressed from different varieties regarding oxidative stability.

On the other hand, oxidative status (peroxide values (PV), anisidine values (AnV) and acid values (AV), indicating the contents of primary and secondary oxidation products and free fatty acids, respectively) and fatty acid profiles of cold-pressed oils from various flaxseed and camelina seed varieties was also determined using official methods.

Also, a linear chemometric method such as principal component analysis (PCA) was used for understanding and visualization of relationships between variables (DSC oxidative parameters, chemical indicators of oxidative status, amounts of fatty acids, radical scavenging activity, color parameters) determined for flaxseed and camelina seeds oils pressed from various cultivars, their characterization, and detection of differences. Thus, chemometric and statistical tools clearly select components of oils impacting their oxidative stability.

The proposed DSC technique in isothermal and non-isothermal modes has proven beneficial in evaluating the changes in oxidative quality of cold-pressed flaxseed, camelina, and hempseed oils during six months of storage (results also presented in A6). The prolongation of oil storage time decreased the DSC parameters, unlike chemical indicators (PV, AnV, Totox and AV), which increased significantly after the storage period. This fact was confirmed by negative Pearson's correlation coefficients for relations between DSC

parameters and chemical indicators and the distribution of these variables on the PCA plot. Moreover, LDA was applied to distinguish fresh cold-pressed oils from deteriorated ones after storage.

On the other hand, the specific shape and number of peaks on crystallization and melting DSC curves affected by the cooling or heating rate can be sufficient to distinguish oils of different fatty acids and triacylglycerol compositions inherent to each oil type. Therefore, discrepancies were observed between crystallization and melting profiles, as well as thermodynamic parameters calculated for flaxseed oils cold-pressed from different varieties (publication A1).

The unique DSC melting patterns were also approached to authenticate cold-pressed flaxseed oils from five cultivars during storage (publication A3), as well as distinguish three cold-pressed oils (flaxseed, camelina, hempseed) from three refined oils (rapeseed, sunflower, soybean) (publication A4). Potential markers of flaxseed oil authenticity among DSC melting curve parameters were selected using X-bar and R-control charts.

Finally, DSC melting curves coupled with four chemometric models (LDA, MARS, SVM, and ANNs) were used for the detection, discrimination, and quantification of cold-pressed flaxseed oil samples adulterated with refined rapeseed oil at four different concentrations (5, 10, 20, 30, and 50%) (results also presented in A5). A shift of characteristic endothermic peaks on DSC melting curves for flaxseed oil to higher temperatures and changes their shape (peak height and area) after the addition of refined rapeseed oil verify the authenticity of flaxseed oil against adulterated samples with rapeseed oils. Regarding the supervised analysis, the proposed classification models correctly detected the adulterations in the test set with high accuracy (95.7 - 99.5%) and precision (87.1 - 98.4%). For accurate identification of adulterated oil samples, the best results revealed the LDA model, whereas in the case of quantification, the best results were obtained for the ANN model with an RMSE = 1.51 and R^2 = 0.996 for the test set. This fact demonstrates the potential of combining DSC data with these classification models as a method for the automated quality control of cold-pressed flaxseed oil.

In addition, chemometric tools such as PCA and OPLS-DA correctly classified the studied oils according to their types (6 classes), technological approach (2 classes), and concentrations of refined rapeseed oil in cold-pressed flaxseed oil (6 classes) (publications

A4 and A5, respectively). Interestingly, the PLS model allowed the most excellent accuracy in prediction quantification of the refined rapeseed oil added to pure flaxseed oil (publication A5).

In the fifth "Conclusions" section, the PhD student summarized in 11 points the conclusions from the obtained results described in 6 publications constituting the reviewed dissertation. The most important conclusions were formulated clearly and unambiguously based on the obtained results, confirming the implementation of the assumed research tasks. An application conclusion was also formulated, which enhanced the rank of the reviewed PhD thesis.

"Table of Figures", "References", and "Keywords" are separate sections located at the end of the commentary on a monothematic cycle of publications constituting a PhD thesis.

The articles' full texts constituting the dissertation and declarations of the percentage contribution of co-authors were included.

The reviewed dissertation was prepared in accordance with good editorial standards, and the cycle of six articles is coherently and correctly ordered. This PhD thesis undoubtedly brings new knowledge about the possibilities of the DSC technique with chemometric methods for the evaluation of the oxidative stability of edible oils and their authenticity. The language is comprehensive and coherent, while errors and inaccuracies are relatively rare. One can find some stylistic and grammar errors in the text of a dissertation.

Nevertheless, during the review, I had a few questions and remarks that should be treated as an opening for a scientific debate, not a thoughtless criticism. They do not affect my high opinion of the PhD thesis. My questions and remarks are presented below:

Major remarks:

- Page 9. Do characteristic values determined using official methods indicate the oxidative stability of oils or their oxidative status?
- Why was the commonly known Rancimat test or other tests not used to determine the oxidative stability of the studied oils? It would be interesting to compare the oxidative stability results obtained with these tests and the proposed DSC technique.

- Page 10. Please justify the choice of the DPPH method to evaluate the antioxidant activity (AA) of the analyzed oils. Why was the AA determination limited to only one analytical method and only for camelina seed oils (publication A2)? Does AA determined by only one analytical method allow an illustration of the total antioxidant potential of oils? It is a pity that individual antioxidants and oxidation products were not determined.
- Page 32, publication A5. What determined the choice of such concentrations (5, 10, 20, 30 and 50%) of refined rapeseed oil for adulteration of cold-pressed flaxseed oil? Unfortunately, I cannot observe any trend in choosing these concentrations. Did the adulteration level of refined rapeseed oil affect amounts of primary and secondary oxidative products, free fatty acids, fatty acid profiles, radical scavenging activity and color of the adulterated flaxseed oils?

Minor remarks that do not require comment during the public defense of the doctoral dissertation:

- Page 1. The style of the articles constituting the doctoral dissertation should be unified and the total impact factor should be verified.
- Page 9. The wavelength for spectrophotometric measurements of anisidine value should be given.
- Pages 16-17. The sentence: "While for camelina oils values were reduced approx. 3.5 times, spanning from 17.0 to 20.7 minutes." does not coincide with the data presented on DSC oxidation curves obtained at 140°C (Figure 3b). Moreover, Figure 3b in the dissertation (page 16) differs from Figure 2b in publication A2. Why? Please explain these discrepancies.
- Page 17. What oil does this sentence refer to: "Additionally, the rate of oxidation, reflecting the speed of the oxidation process, was calculated from the DSC oxidation curve, at 120 °C, this rate ranged from 0.02 to 0.03 W/h·min, while at 140 °C it was in the range from 0.08 to 0.09 W/h·min."
- Page 18. Figure 4. It is worth paying attention to the significant figures of the determination coefficient, R^2.
- Page 20. Is the unit (1/min) for the $t_{1/2}$ parameter correct?

- Page 20. It is not very accurate to use the term "the most stable cultivars".
- Page 28. This sentence: "Figures 10 (a–b) demonstrates control charts for main peak height and area parameters (h2, A2, and PA2), which exhibited decreasing trend throughout the oil's shelf life." is not correct due to Figures 10 (a–b) demonstrate only control charts for main peak height (h2) and area parameters (A2). The X-bar control chart for a percentage of the second peak area (PA2) was not presented in PhD thesis (it was depicted in publication A3).
- Page 29. This sentence: "Moreover, the ratios calculated from the DSC parameters were also analyzed using control charts, for which decreasing or increasing trends were observed as it was shown in Figures 5 and 6 of the article (A3)." requires clarification. X-bar control charts of ratios calculated from DSC melting curves were shown only in Figure 6 of article A3.
- References to the descriptive part of the dissertation should be presented in a uniform style.
- Publication A1, Figure 1. The peak temperatures from the DSC crystallization curves in Figure 1 do not coincide with the values in Table 3 and the text (subsection 2.3.). Is Figure 1 prepared correctly?
- Publication A1. Title of subsection 2.5. is not correct.
- Publication A2, Figure 3b and 6b. Incorrect figure legend; should be "OET" and "T_{end}"
- Publication A3, subsection 3.1.2. There are no units for PV and AV results.
- Publication A5, pages 9 and 13. The paragraphs regarding the description of the ANN model are identical. Was it possible to avoid repetition?

4. Final assessment of the dissertation

In the assessment of the dissertation, I conclude that it presents a very good scientific level. PhD student proved her understanding of the state-of-the-art in the undertaken research area concerning the chemistry and technology of fats. She can carry out research independently, which is one of the main requirements for obtaining a PhD. Mahbuba Islam demonstrated her skills and knowledge in data reduction, analysis, modelling and interpretation. All carefully performed experiments were well arranged, and analytical methods were correctly applied. The experimental results are presented clearly and with

the appropriate chemometric and statistical analysis. The methodology is sound and fulfils the standards of good scientific practice. I would like to emphasize the practical aspect of this work strongly. The proposed DSC is a prominent analytical technique to evaluate the quality of oils and fat and their possible adulteration, thereby offering potential applications in quality control within the fat industry.

Final evaluation statement

The PhD thesis it reviews is of the international standard. I am sure that the doctoral dissertation of Mahbuba Islam fully complies with the conditions set out in Art. 187 of the Law on Higher Education and Science in Poland of July 20, 2018 (in Polish: Prawo o szkolnictwie wyższym i nauce, Dz.U. z 2018 r. poz. 1668 ze zm.) In view of the above, I am applying for admission of mgr Mahbuba Islam to the next stages of the doctoral dissertation defense.

Application for distinction

Taking all the above mentioned, mainly: (1) novelty in the scope of the discussed subject; (2) results with practical importance obtained by the simple, reliable, and convenient DSC technique offering qualitative and quantitative information within a short time with good reproducibility; (3) application of chemometric methods to solve problems of classification and prediction results; (4) high quality of the PhD thesis confirmed by 6 publications in journals from the JCR list (total IF = 25.5 and total MEiN = 700); (5) very good academic achievements (co-author of 13 publications, in 8 of them, she was the first author, total IF = 49.5 and total MEiN = 1460), I state that the PhD thesis prepared by Mahbuba Islam should be awarded by distinction.

Prof. dr. hab. Aleksandra Szydłowska-Czerniak

Aleksandra Szydłowska-Czerniak