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### **Review Report on the doctoral thesis of Mahbuba Islam MSc**

entitled “The application of Differential Scanning Calorimetry (DSC) for the quality assessment of selected edible oils”

Supervisor: Assoc. Prof. Jolanta Tomaszewska-Gras PhD;

Co-Supervisor: Anna Kaczmarek PhD, Eng.

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#### **Legal basis for the review**

- Act of 20 July 2018 – The Law on Higher Education and Science (Journal of Laws of 2020, item 85 as amended);
- Resolution of the Discipline Council for Food Technology and Nutrition, Poznań University of Life Sciences of 19 October 2023 regarding appointing me as a reviewer of the doctoral dissertation (Letter of the Chairman of the Discipline Council for Food Technology and Nutrition, Prof. Magdalena Rudzińska, PhD, Poznań University of Live Science of 26 October 2023).

#### **Evaluation of the original solution to the academic problem**

Unhealthy eating habits and low food quality, together with stress, a lack of physical activity and environmental pollution, are the main causes of lifestyle diseases such as obesity, diabetes, hypertension, atherosclerosis and cancer. But in recent years, thanks to growing consumer awareness, the demand for high-quality food with exceptional nutritional value, rich in active ingredients contributing to the prevention of lifestyle diseases has increased.

Such products undoubtedly include edible oils, especially cold-pressed ones, which furnish a significant portion of essential fatty acids and other functional compounds. The lipid fraction of many plants is rich in bioactive compounds and is a desirable component of the human diet. Vegetable oils contain a high content of essential polyunsaturated fatty acids (PUFAs) which play a many important biological roles in the human body. They determine the structure of cell membranes, limit triglyceride synthesis, regulate insulin secretion and serve as substrates for the production of eicosanoids.

Obtaining edible oils using the cold-pressing method preserves a remarkable combination of acylglycerols, along with an array of other bioactive compounds such as phenolic compounds, sterols, tocopherols, tocochromanols, phospholipids, carotenoids and pigments. The high content of PUFAs in edible oils makes them vulnerable to oxidative degeneration, and the relatively high price of cold-pressed oils contributes to potential attempts to adulterate these products with other oils. Most methods for assessing the quality of edible oils are based on time-consuming chemical analyses, requiring the use of significant amounts of organic reagents, often toxic. Therefore, developing techniques that allow for a quick and clear assessment of the authenticity and quality of cold-pressed oils during their shelf life is crucial.

Taking the above into account, the selection of research issues in the doctoral thesis, specified as: " investigating the possibility of using the instrumental technique of differential scanning calorimetry (DSC) for the comprehensive characterization of cold-pressed edible oils, i.e. flaxseed, camelina and hempseed oil, obtained from different cultivars, in terms of assessing oxidative stability and its changes during storage as well as the possibility of assessing authenticity of oils and detecting adulteration with refined oils" I consider as justified, interesting, scientific and innovative.

The research presented in the doctoral dissertation was funded by the National Science Centre, Poland, grant number: 2018/31/B/NZ9/02762, entitled: "Identification of lipidomic biomarkers for recognition of the authenticity of edible oils supported by DSC profiling and chemometrics".

### Structure of the thesis

The reviewed Ph.D. thesis of Mahbuba Islam is based on the coherent collection of the 6 scientific articles published in international journals:

1. Tomaszewska-Gras, J.; **Islam, M.**; Grzeca, L.; Kaczmarek, A.; Fornal, E. Comprehensive Thermal Characteristics of Different Cultivars of Flaxseed Oil (*Linum usittatissimum L.*). *Molecules* 2021, 26, 1958. <https://doi.org/10.3390/molecules26071958>; output 51%
2. **Islam, M.**, Muzolf-Panek, M., Fornal, E. et al. DSC isothermal and non-isothermal assessment of thermo-oxidative stability of different cultivars of *Camelina sativa L.* seed oils. *J Therm Anal Calorim* 147, 10013–10026 (2022). <https://doi.org/10.1007/s10973-022-11367-8>; output 45%
3. **Islam, M.**; Kaczmarek, A.; Grygier, A.; Tomaszewska-Gras, J. DSC Phase Transition Profiles Analyzed by Control Charts to Determine Markers for the Authenticity and Deterioration of Flaxseed Oil during Storage. *Foods* 2023, 12, 2954. <https://doi.org/10.3390/foods12152954>; output 45%
4. **Islam, M.**, Montowska, M., Fornal, E., Tomaszewska-Gras, J. (2023). Discrimination of Selected Cold-Pressed and Refined Oils by Untargeted Profiling of Phase Transition Curves of Differential

Scanning Calorimetry. Polish Journal of Food and Nutrition Sciences, 224-232. <https://doi.org/10.31883/pjfns/169425>; output 45%

5. **Islam, M.**; Kaczmarek, A.; Montowska, M.; Tomaszewska-Gras, J. Comparing Different Chemometric Approaches to Detect Adulteration of Cold-Pressed Flaxseed Oil with Refined Rapeseed Oil Using Differential Scanning Calorimetry. *Foods* 2023, 12, 3352. <https://doi.org/10.3390/foods12183352>; output 45%
6. **Islam, M.**, Kaczmarek, A. Tomaszewska-Gras, J. Differential scanning calorimetry as a tool to assess the oxidation state of cold-pressed oils during shelf-life. *Journal of Food Measurement and Characterization* (2023). <https://doi.org/10.1007/s11694-023-02152-8>, output 51%.

The Total Impact Factor of these publications is very high, equal to IF = 26.1 (Total MEiN: 700). The PhD student is the first author in 5 from those 6 publications. Her individual output is in the range of 45 – 51 %.

The PhD dissertation has 49 pages (+ reprints of articles) and is written in English. The dissertation consists an abstract in English and Polish, a list of publications constituting the basis of the doctoral thesis, an introduction, a materials and methods description, research goals and hypotheses, results as a description of the content of publications, conclusions, figures (14), references (82 items) and attachments in the form of reprints of articles chosen as the basis for the PhD procedure.

The thesis structure is correct and contains all the necessary elements of a doctoral dissertation based on coherent collection of published articles.

### **Evaluation of the merits of the dissertation**

This thesis is concerned with the possibility of using the instrumental technique of differential scanning calorimetry (DSC) in the comprehensive characterization of oils in terms of oxidative stability and authenticity assessment.

The first part of the thesis provides background on the subject and an overview of the six papers that constitute the substantive part of the thesis. The remainder of the thesis consists of the six papers. All publications included in the dissertation are characterized by a very high scientific level and care for appropriate presentation and discussion of the presented results and conclusions. The articles were published in renowned international journals, so they were reviewed by experts in the presented field, which confirms the high scientific level of the publications.

In the article A1 the thermal properties of selected cultivars of flaxseed oils were described by the use of the differential scanning calorimetry (DSC) technique. The crystallization and melting profiles of oils were analyzed isothermally at different scanning rates. The oxidative induction time (OIT) isothermally at 120 °C and 140 °C and oxidation onset temperatures (Ton) at 2 and 5 °C/min scanning rates were measured.

It has been shown that the scanning rate has a significant influence on the behavior of the oil during melting. The melting curves of the selected cultivars of cold-pressed flaxseed oils at a

heating rate of 5°C/min did not differ, which can be used in profiling analyzes to assess the authenticity of linseed oil. Flaxseed oil is very susceptible to thermal oxidation, it has been observed that its stability decreased with increasing temperature and decreasing heating rate. Then, the relationship between DSC oxidation stability parameters and chemical indicators of oxidative stability and the fatty acid (FA) composition of oils was assessed. Significant negative linear correlations were found between unsaturated FA content (C18:2, C18:3 n-3) and DSC parameters (OIT, Ton). Principal component analysis (PCA) established a strong correlation between total oxidation value (TOTOX), peroxide value (PV) and all DSC parameters of thermo-oxidative stability.

Next, the thermal resistance to oxidation of three different cultivars of cold-press camelina oil i.e., Omega, Luna and Śmiłowska by means of isothermal and non-isothermal differential scanning calorimetry (DSC) oxidation measurements were tested (article **A2**). The characteristics of the tested cold-pressed oils were supplemented with chemical analysis i.e., FA composition, peroxide value, p-anisidine value, acid value and radical scavenging activity by 2,2-diphenyl-1-picrylhydrazyl (RSA DPPH).

In the study comparing the oxidative stability of these three varieties of camelina, the influence of heating temperature (isothermal DSC analysis) on the oxidation induction time (IOT) and oxidation rate was checked. The correlation analyzes performed showed, as in the case of linseed oil (**A1**), a statistically significant positive correlation between the content of some FAs, especially  $\alpha$ -linolenic acid (C18:3, n-3) and DSC oxidation parameters (OIT and Ton). The oxidative stability of oils from camelina were also highly positively correlated with value  $b^*$  (yellowness, resulting from the content of color antioxidant compounds like carotenoids) and the radical scavenging activity by 2,2-diphenyl-1-picrylhydrazyl (RSA DPPH).

The DSC isothermal and non-isothermal tests were also used for the assessment of the oxidative stability of three cold-pressed oils during six-month storage (**A6**). For this research, three popular cold-pressed oils i.e., flaxseed oil, camelina seed oil and hemp seed oil were studied. Studies have shown that DSC parameters obtained from isothermal (OIT) and non-isothermal (Ton) measurements changed corresponding to the chemical indicators ( PV, p-AV, TOTOX, AV) during the shelf life of oils. Important information presented in the work is the indication of the optimal test for monitoring the deteriorative changes in oils during storage (DSC isothermal test at 120 °C).

The next work (**A3**) focused on the effect of storage time on the DSC melting curves of different cold -pressed flaxseed oil varieties was investigated in the order to assess the stability of thermodynamic parameters and understand how storage time affects the melting behavior of oil samples.

Using the deconvolution analysis procedure, which enabled the data to be collected at peak temperature (T), peak height (h), the peak area (A), and the percentages of the area (PA), as well as the ratio calculated from these parameters four characteristic peaks of phase transitions at

around  $-36$ ,  $-30$ ,  $-25$ , and  $-12$  °C were identified. It was shown that during storage there was a significant decrease in peak height ( $h_2$ ) and area percentage (PA2) for the second peak of the melting profile, therefore they were considered as indicators of oil degradation. Whereas DSC parameters for the first peak and the third peak, changes were statistically not significant within storage ( $p > 0.05$ ); thus, they can be used as markers of flaxseed oil authenticity. The novelty of this study was the use of X-bar and R control charts as statistical process control tools to identify markers of oil authenticity and deterioration.

The possibility of using entire DSC melting profiles as a kind of unique fingerprint for assessing the authenticity of edible oils has also been demonstrated in subsequent studies (A4). The DSC profiles combined with advanced chemometric methods, mainly orthogonal partial least squares-discriminant analysis (OPLS-DA) were used in order to distinguish cold-pressed oils (flaxseed, camelina, hempseed) from refined oils (rapeseed, sunflower, soybean).

The usefulness of Differential Scanning Calorimetry (DSC) in the broadly understood quality assessment and identification of vegetable oils was also demonstrated in Article 5. The paper presents the analysis of DSC melting curves of various flaxseed oil cultivars, both pure and adulterated with refined rapeseed oil at different concentrations.

In order to assess the usefulness of the DSC technique for detecting adulterations, three chemometric approaches were compared: classification models, regression models, and a combined model of orthogonal partial least squares discriminant analysis (OPLS-DA). These techniques were used to construct a classification model to categorize the level of adulterant in the oil and a regression model to quantify the concentration of the adulterant.

#### Summary :

The dissertation has a very contemporary and modern aspect. It presents a multi-faceted approach to the possibility of using the instrumental technique of Differential Scanning Calorimetry (DSC) for the comprehensive characterization of cold-pressed edible oils in terms of assessing oxidative stability and its changes during storage, as well as the possibility of assessing the authenticity of oils and detecting adulteration with other oils.

The multidisciplinary nature of the work and a very wide scope of research methodology should be emphasized, including the DSC oxidation test, DSC melting and crystallization phase transition profiles, determination of chemical indicators of oils quality, the use of X-bar and R control charts as statistical process control tools to identify markers of oil authenticity and deterioration, and the use of various classification and regression chemometric models to detect adulteration of oils. The obtained results are of practical importance and contribute to the progress in the application of DSC techniques for the quality assessment of edible oils.

#### **The specific questions**

Q A1: What was the purpose of the color measurement of oils?

Q2: The main idea of the research described in the articles (A4 and A5) was to use DSC as an effective analytical technique to distinguish cold-pressed oils from refined oils and to detect adulteration of cold-pressed oil with other types of refined oil. What is the opinion of the PhD student about the possibility of using DSC to identify cold-pressed and refined oils from the same plant? Can refining vegetable oil have a real impact on DSC melting curves to the extent that it will cause an error in assessing the authenticity of the oil?

Q3: What is the opinion of the PhD student about the possibility of creating a database of DSC phase transition profiles of edible oils as a unique fingerprint for each type of oil? What DSC analysis conditions, statistical analyzes and/or chemometric methods should be taken into account to optimize the level of success in oil identification?

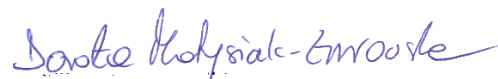
### **Summary**

I hereby declare that the doctoral dissertation entitled "The application of Differential Scanning Calorimetry (DSC) for the quality assessment of selected edible oils" written by Mahbuba Islam M.Sc. meets the requirements for doctoral dissertations according to the Act of 20 July 2018 – The Law on Higher Education and Science (Journal of Laws of 2020, item 85 as amended), and I recommend that the Discipline Council for Food Technology and Nutrition, Poznań University of Life Sciences, to admit Mrs. Mahbuba Islam M.Sc. for the public defense of the dissertation.

### **Application for distinction of doctoral thesis**

Taking into account the originality of the solution to the scientific problem, the cognitive and application value, and the very high scientific activity of the PhD student, I apply for the distinction of the reviewed doctoral dissertation because:

- In my opinion, the reviewed doctoral dissertation significantly exceeds the average level of doctoral thesis in terms of the importance of the problem solved, the quality and scope of the research conducted, and the significance of the results obtained.
- Doctoral dissertation introduces novelty in the scope of the discussed subject.
- It shows a very good quality of the PhD thesis confirmed by 6 publications in highly recognized research journals (Total Impact Factor IF = 26.1).
- MSc Mahbuba Islam is co-author of 13 scientific articles in peer-reviewed journals from the JCR list. Among those 13 articles, she is the first author for 8 of them, published in journals with IF > 4 (IF 4.9, and 5.2).



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