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Review Report on the PhD thesis

Author: **Qian Ying, MSc Eng.**

Title: **The composition of triacylglycerols as an indicator of edible oil adulteration**

Supervisor: **Prof. dr. hab. Magdalena Rudzińska**

Academic supervisor: **Dr. inż. Arkadiusz Majewski**

1. Project background

Edible vegetable oils are widely used as staple foods and ingredients that are included daily for flavouring and cooking food at home and in industrial food manufacturing. Edible oils induce satisfaction and supply nutrients such as unsaturated fatty acids, phytosterols, antioxidants and other bioactive compounds, which are crucial for human health and well-being. There are many kinds of vegetable oils on the Polish market today, ranging from traditional and generally inexpensive rapeseed, sunflower, and soybean oils to more expensive oils such as peanut, flaxseed, sesame, olive, black cumin, and edible niche oils. For this reason, adulteration of expensive oils can be very beneficial for raw material suppliers, oil manufacturers and distributors. Oil adulteration with cheaper vegetable oils, substitution, purposeful misnaming, and counterfeiting are frequently associated with economic incentives, such as lessening production costs or facilitating enhanced shelf life. On the other hand, adulteration aims to develop the professed oil quality and imitate a recognized brand. These fraudulent activities are a serious problem because they reflect the deception of consumers and can lead to adverse health and safety effects. The safety and quality of edible vegetable oils are significant and alarming challenges in fat inspection, particularly concerning

the recognition of oil kind, its authenticity, and oxidative deterioration. Therefore, various analytical techniques, including chromatographic and spectroscopic methods, have been applied to detect adulteration in edible vegetable oils. The combination of these analytical methods with chemometrics tools, such as neural networks that are regarded as intelligent modelling systems, can be used to obtain better results and solve complex problems of oil authenticity.

The PhD thesis of Qian Ying is devoted to evaluating the ability of an artificial neural network (ANN) to detect fraud in vegetable oils based on a few indicator molecules such as fatty acids, triacylglycerols and sterols. Chromatographic methods such as gas chromatography with flame ionization detection (GC-FID), high-temperature gas chromatography with FID (HTGC-FID) and high performance liquid chromatographic with evaporative light scattering detection (HPLC- ELSD) were applied to reliable quantitative analysis of fatty acids, triacylglycerols and sterols in eight kinds of vegetable oils (371 oil samples). By using chromatographic methods, characteristic components of oils can be quantified, while multivariate data analysis provides better insight into these compounds associated with each type of oil for classifying and identifying different levels of fraud. Thus, this PhD thesis addresses the highly vital areas of current research on the identification of adulterants in edible oils.

2. General description of the doctoral thesis

The reviewed PhD thesis presents investigations on (1) the application of chromatographic methods for the determination of fatty acids, triacylglycerols and sterols in 371 oil samples provided by suppliers or purchased in Polish markets; (2) the utilization of the principal component analysis (PCA) to reduce the dimension of high-dimensional data to lower-dimensional space for the development of three artificial neural networks (ANNs); (3) the employing two techniques of neural networks such as a multilayer perceptron (MLP) and radial basis function (RBF), to detect the adulteration of the studied vegetable oils; (4) the establishment of MLP to identify black cumin oil adulteration. In general, triacylglycerols, as the main components of vegetable oils, can be used as indicators for identifying their adulteration. The MLP ANN model based on 6

triacylglycerols and 15 neurons in the hidden layer achieved the highest accuracy and correctly identified adulteration of eight oil types with a threshold of 20%.

The use of major classes of compounds, including fatty acids, triacylglycerols, and sterols treated with chemometric analysis, as markers for vegetable oil adulteration are highlighted in the reviewed PhD thesis.

This PhD thesis is well structured and correctly presented. It consists of abstracts in English and Polish (4 pages), a list of abbreviations (2 pages), and seven coherent chapters (65 pages), including 1. Introduction, 2. Literature review, 3. Aims of the thesis, 4. Materials and methods, 5. Results and discussion, 6. Summary and 7. Conclusion, a list of 90 references arranged alphabetically (13 pages), acknowledgements, a list of 20 tables and 15 figures (2 pages) and 27 tables in an appendix (42 pages). The dissertation is written in English.

The second chapter, entitled "Literature review", consists of a literature review with a logical partition. This section provides the necessary information about the main components of vegetable oils, namely triacylglycerols, fatty acids and sterols. Moreover, typical edible oil processing, oil adulteration and economic data on edible oils were described. The literature part ends with a chapter on neural networks as mathematical models used to estimate the quality of vegetable oils. The literature for this section is appropriately referenced. However, the citations on fatty acids and sterols compositions for niche oils not studied in the experimental section are not justified. It is worth mentioning that most of the references are from the last decade, showing the topical issues. This part is correctly written and provides a good background for understanding the rest of the thesis.

The third chapter is the outline of the dissertation, which highlights the thesis objective along with the list of the seven main tasks.

The main experimental part of the thesis (the fourth chapter: "Materials and Methods", and the fifth chapter ", Results and discussion") exhibits the same structure and is divided into sections, which are typical for scientific articles. Chapter 4 is dedicated to describing the used materials, chemicals, and procedures of the applied analytical methods for the quantitative analysis of fatty acids, triacylglycerols and sterols as well as applied chemometric tools like principal component analysis (PCA) and artificial neural

networks (ANNs). Chapter 5 is focused on the obtained results of fatty acids, triacylglycerols and sterols and their discussion. Moreover, the regression ANNs with various topologies based on the results of the chosen analytes were developed as powerful and simple tools to detect and quantify adulteration of vegetable oils. Finally, the most robust models for quantifying the amount of adulteration in black cumin oil with soybean oil were tested. The advantage of this part is that only the average analyte results are presented in tables 5, 7 and 8, while the amounts of fatty acids, triacylglycerols and sterols in each studied oil sample are listed in the tables included in the appendix.

At the end of the dissertation, the summary and general conclusions of the performed research were described in the sixth and seventh chapters, respectively.

The reviewed PhD thesis is prepared with good editing standards. All tables, figures and schemes are carefully prepared and clearly presented. The language is comprehensive and coherent, while errors and inaccuracies are relatively rare.

3. Critical remarks and specific comments

Even though the PhD thesis was written correctly, there are some major and minor errors to correct. Below some mistakes and inaccuracies are pointed out:

3.1. Major remarks:

Title

- The title is not adequate for the content of the dissertation. It seems too specific and sounds like one point of the conclusions. I understand that the determination of triacylglycerol compositions of edible oils was only one of the research tasks, and the selected triacylglycerols were the input data for the three developed ANN models. However, I found much more analytical data in the thesis, such as profiles of fatty acids and sterols, than the triacylglycerol compositions alone. The title definitely lacks information on the used chemometric tools, namely the ANNs.

Chapter 2: "Literature review"

- Due to the fact that triacylglycerols are esters derived from glycerol and three fatty acids, would it not be justified to start chapter 2 with the characterization of fatty acids and then move on to the definition and analysis of triacylglycerols in vegetable

oils? Correctly, chapter 5, "Results and discussion", begins with presenting the fatty acid results for the investigated oils.

- Obviously, genetic, agronomic, environmental, and analytical parameters of the applied methods affect the fatty acid and sterol profiles of vegetable oils. Why however, the doctoral student supported this fact with citations for niche and unconventional oils, not analyzed in the experimental part (pages 11-13). In my opinion, the references are not properly selected.
- The sentence is unclear to me: "Edible vegetable oil can be produced by pressing or refining." Mechanical pressing and chemical extraction are basic methods for obtaining crude vegetable oils. Refining is practically mandatory for crude oils that cannot be consumed as virgin oils to provide a product with an attractive appearance, a neutral taste, and more resistance to oxidation.
- What is the justification for including subsection 2.3 "Economic data on edible plant oil", in the dissertation? This subsection should concern the production costs of oils and their prices in terms of adulteration of more expensive oils with cheaper substitutes.
- Subsection 2.4 "Methods of adulteration of edible oils": The literature review of different analytical techniques (not only chromatographic methods) for the identification of adulterants in edible oils would be needed for a better understanding of this problem.
- In subsection 2.5 "Artificial neural networks (ANN)", there are no literature examples of the application of analytical methods in combination with chemometric tools (mainly neural networks) to estimate the authenticity of vegetable oils. **This subsection should end up with the scientific novelty of the doctoral thesis.**
- **It is a pity that the novelty of this project and practical applications were not highlighted.**

Chapter 3: "Aims of the thesis"

- Laconic research tasks were bulleted in this chapter. What does "evaluated" mean? Were the chromatographic methods validated? Can the purchase of oils qualify as a research task?

Chapter 4: "Materials and Methods"

- Why were not the oil samples divided into those purchased in Polish markets and supplied by factory labs? Why were not analysed commercially available soybean oils? (page 25).
- Each analytical method procedure ends with the sentence: "Each sample was determined in duplicate". Why were not the calculated standard deviations added to the results of fatty acids, triacylglycerols and sterols in the studied oils (appendix, tables 10.1-10.27)? Are there averages of two measurements of each analyte in these tables?
- What are the validation parameters of the applied analytical methods? Can the HPLC-ELSD method be used to determine triacylglycerol composition in each investigated oil (this method was only used to analyze the triacylglycerol profiles for linseed oils)?

Chapter 5: "Results and Discussion"

- What was the statistical test used to find no significance (pages: 33 and 43)? Did not the obtained results of fatty acids in the analyzed oils differ significantly (page 33)?
- Literature examples of squalene content in niche and unconventional oils should not be cited (page 45) and compared with the obtained results for studied oils.
- What are the valid inputs for Model 2? The results summarized in table 11 do not coincide with those mentioned in the text (except for "Conclusions" point 8). There are inconsistencies in the selection of fatty acids for Model 2 throughout the dissertation - pages: 2 (abstract in English and Polish), 53, 59 (caption under table 17), 62 (table 19), and 65.
- Why was only black cumin oil and soybean oil selected for testing ANN models?

Chapter 7: Conclusion

- Why could the GC-FID method be used to determine fifteen triacylglycerols in vegetable oils? Table 7 summarizes the results of 28 triacylglycerols in the analyzed oils.
- In which oils were 17 sterols determined? The applied analytical method allows the determination of 19 sterols (see table 8).

3.2. Minor remarks:

- In the “Abstract”, abbreviations are used without their expansion.
- The division of the list of abbreviations into "Abbreviations for FAs" and "Abbreviation for TAGs" is incorrect and unnecessary. In the section "Abbreviation for TAGs" there are abbreviations of fatty acids and not triacylglycerols. The "Abbreviation" section does not contain all the abbreviations used in the dissertation. Why?
- Some abbreviation expansions are incomplete; for example should be: gamma-Linolenic acid.
- The lack of consistency in using abbreviations was noticed. Although the acronym was created, it was not applied.
- The singular and plural abbreviations should be created and used throughout the dissertation (e.g. FA for fatty acid and FAs for fatty acids; TAG for triacylglycerol and TAGs - triacylglycerols etc.).
- Some typing errors were found; for example: "roman spectroscopy" and "deep leaning algorithms" (page 4), „machine-earning” (page 20).
- What are the differences between "rapeseed oil" and "canola oil" (page 9)?
- Figure 4 (page 24). Abbreviations of the applied analytical methods should coincide with those used in the text.
- Each used equipment should be characterized in detail (name, manufacturer, country).
- The title "Using high-performance liquid chromatography" should be moved to next the page.
- The extraction of sterols from oils should be briefly described (page 28).
- More details about the Statistica software should be provided (page 28).
- What values (standard deviation or confidence intervals) are presented with the means listed in tables 5, 7 and 8 (pages 34, 41-42, 46-47)? This should be explained in the table footnote. Are they averages of the number of tested oils, or is duplicate analysis for each sample taken into account?
- The results in the text, tables and figures should not differ in the number of significant figures.

- Better be careful about mentioning the full name of the company providing the oil samples (page 33).
- I suggest not using the terms "we" and "our" in the PhD thesis. Only a PhD student is the author of this thesis.
- What does the term "normal sunflower oil" mean (pages 35 and 44)?
- Flagella et al., 2002 did not determine folic acid in sunflower oil (page 35).
- What was C18:0 content in linseed oil? Should this value be 2.74% in the text (page 35) or 3.79% in table 5?
- In the sentence: "The SFAs in linseed oil (making up 14.71%) primarily ..." the term "linseed oil" should be replaced with "black cumin oil" (page 36).
- The range of the relative retention time (rrt) given on page 37 is appropriate only without the rrt value for trinonadecanoyl-glycerol.
- "NNN was used as an internal standard and was eluted last (Figure 8)." Why was not the abbreviation "NNN" quoted in figure 8 (pages 38-40)?
- There is no description of the axis in figures 7-9. Why?
- Figure 9 should be moved to page 43 after explaining the necessity of using the HPLC-ELSD method to determine the triacylglycerol profiles of linseed oils.
- It is a pity that the analyzed oils were not listed in the same order in tables 5, 7 and 8.
- This phrase, "the greatest number of TAGs were detected in soybean and linseed oil, with 16 and 17, respectively." (page 43), is unclear. As can be seen, 16 triacylglycerols were determined in both soybean and linseed oils (table 7).
- There is no agreement between the results of triacylglycerol amounts in sunflower oil presented in the text (page 44) and tables 7 and 10.16. Why?
- The reference to Appendix Table 10.13 (page 45) is incorrect. It should be Appendix Table 10.21.
- Is the range of 49% to 72% correct (page 54)?
- The text was incorrectly assigned to tables 12 and 13 (page 55). It should be table 13 and table 14, respectively.
- Reference style should be the same for each item.

The doubts mentioned above do not significantly impact the valuable material presented in the dissertation. Hard and creative work and the ability to use neural networks allowed solve experimental problems and accurately predict oil adulteration. Moreover, the reviewed thesis presents scientific novelty because the combination of process analytical tools with chemometrics offers robust techniques that can play an important role in the fat industry.

4. The summary and general conclusions

To summarize, the reviewed PhD thesis represents high-level scientific work. It is evident that Qian Ying has sufficient knowledge and understands problems which can appear in the analysis and technology of edible oils. All experiments are well arranged, whereas measurement techniques and methods are correctly and competently applied. The explanations and interpretation of the obtained results are suitable and focused on the relevant topics. The thesis is very interesting, easily and pleasantly to read due to its high standard of literary quality. The conclusions and implications are clearly linked to the nature and contents of the research framework and findings. Therefore, it seems to be an interesting topic for scientists working on the chemistry and technology of oils and fats.

Final evaluation statement

The PhD thesis it reviews is of the international standard. I am sure that the doctoral dissertation of Qian Ying 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 Qian Ying to the next stages of the doctoral dissertation defense.

Prof. dr. hab. Aleksandra Szydłowska-Czerniak

Aleksandra Szydłowska-Czerniak