REVIEW

Review of the Ph.D. dissertation by Xi He, MSc, Eng.
entitled: *Gas Chromatography and Combination Techniques in the Assessment of Authenticity and Quality of Baijiu Alcohols*
carried out at the Laboratory for the Study of Volatile and Sensory Active Compounds of the Department of Plant Food Technology
Faculty of Food Science and Nutrition of the University of Life Sciences in Poznań
Supervisor: Prof. Henryk H. Jeleń

The broadly understood quality control of products intended for consumption includes various analytical methods allowing to determine the composition and content of the main ingredients, the ingredients covered by the manufacturers' declarations, as well as the acceptable and unacceptable impurities of the product. In process analytics, as well as in the final product inspection, various quality methods play an increasing role. They can be seen as a focus on progressive automation, competition of market food producers, efficient methods of classifying the quality of food products and lowering costs. More or less advanced methods of instrumental analysis, combined with methods of quantitative analysis, allow precise determination of the content of food ingredients. In turn, qualitative analysis methods together with classification procedures using chemometric data processing, allow to assess the composition of the product.
Currently, among the most commonly used techniques for isolating volatile compounds from food samples, are the techniques based on *headspace analysis*, in which compounds released from the sample and located in the gaseous phase over the tested product are directly analyzed. Due to the simplicity and low or no interference in the chemical composition of the tested product, these methods are particularly valued in the analysis of fragrance compounds. Among these types of techniques, the most commonly used, due to its versatility and universality, is the solid phase microextraction (SPME) technique, in which volatile compounds are sorbed on a fiber covered with an ad/absorption phase, where their adsorption takes place. Such a fibre may be directly introduced into the gas chromatograph dispenser where the analytes are desorbed. At the same time, new techniques are still being sought to simplify and shorten the analysis time. These techniques make it possible to simultaneously obtain low limits of quantification, as well as the highest selectivity and resolution (in accordance with Purnell's concept). With the development of complete two-dimensional gas chromatography (GCxGC), there are more and more applications devoted to the profiling of volatile compounds by this technique for the identification of geographical or botanical origin. This also makes it possible to differentiate food products in terms of a specific aroma – important from the point of view of consumer preferences.
All the above-mentioned issues are the basis for the creation of the reviewed dissertation. The doctoral student decided to develop new analytical methods to assess the authenticity and quality of Baijiu – a traditional Chinese spirit drink. The unique way of obtaining this drink results in the fact that it is characterized by the richness of volatile compounds.

Existing traditional variants of carrying out the fermentation process cause differentiation due to the main aroma. The complexity of the composition of the product requires advanced analytical and chemometric tools to identify as many volatile compounds as possible, characterizing the product in terms of aroma, geographical and botanical origin. Like many alcoholic products, Baijiu is counterfeited, among others, through the addition of water or other cheaper products. Botanical and geographical authenticity are also subject to counterfeiting, despite the certification of the product as a regional food with a protected designation of origin. Therefore, I believe that the task set by the PhD student and her supervisor is fully justified for both cognitive and application reasons. This topic is certainly in the mainstream of original and current research problems.

The dissertation submitted for assessment consists of 4 scientific and research papers published in specialized journals from the so-called "*JCR list*", whose *impact factor* (IF) ranges from 2.216 to 9.231. The whole was preceded by an abstract in Polish and English, as well as an introduction prepared in English. This introduction provides a description of the set objectives of the work along with a concise commentary on the results achieved as a result of the implementation of the dissertation together with the bibliography (41 pages in total). The work is crowned with statements of co-authors about the contribution made to the creation of each publication. Analyzing the presented copies of the publication, I find that one of them is a two-author (Ph.D. student and her Supervisor), two of them have three authors assigned, and one of them is a five-author. The Ph.D. student is the first author in all the presented works. This clearly indicates her dominant participation in the preparation of this study and in the implementation of studies included in the research schedule. Multi-authored publication, in turn, proves the ability to work as a team, as well as to draw and adapt the experience of specialists to her own research goals. It is a pity that the PhD student did not present a summary of her entire body of work: publications, chapters, poster messages, oral presentations and possible data on scholarships awarded, internships or funds obtained for conducting research projects. It is difficult for me to assess the scientific activity of the PhD student without this information. In addition, it would be reasonable to provide, in the statements of the authors, in addition to a descriptive presentation of the involvement in the creation of the publication, a more detailed participation of individual members of the team implementing the tasks set out. The Ph.D. student should be commended for a list of abbreviations and acronyms useful for nomenclatural reasons, at the beginning of the report.

The theoretical part, i.e. the description of world literature, is a synthetic content that introduces the reader to the issues related to the subject of the dissertation. The Ph.D. student characterized the material of her research by presenting the technologies of obtaining, giving the nomenclature and characteristics of the Baijiu product depending on the type of raw material and the conditions of the fermentation carried out. She paid a lot of attention to the analytical methods used in the qualitative and quantitative identification of the ingredients constituting the compositions of this drink. Extremely valuable and interesting data can be found in Table 2, compiling an overview of the procedures for assessing the authenticity of distilled alcohols together with the measured parameters. In the further part of the paper, the Ph.D. student presents literature data on analytical techniques used in the Baijiu analysis – among them GC-FID, GCxGC-TOF/ MS, e-nos (based on appropriate sensors or SPME/MS) or IR/MS, listing their advantages and disadvantages. I can only state that this text is an interesting reading, well referring to the rest of the dissertation and introducing the reader to further issues in a very understandable and competent way.

Getting acquainted with the theoretical introduction prepared in this way fully justifies the statement that the Ph.D. student gained a perfect insight into the applied research techniques, keeps track of the literature on the subject, and thanks to this, the concept of the research carried out as part of the doctoral dissertation corresponds to the latest literature trends and methodological expectations in the area of assessing the authenticity of Baijiu alcohols. At this point, I would like to mention that after reading this part of the dissertation, my knowledge about the production of traditional Chinese Baijiu has increased significantly. I wish it was not possible to taste it.

Undoubtedly, I believe that the **research objective** set by the dissertation Supervisor and the Ph.D. student, which was to determine the authenticity and quality of Baijiu alcohols using selected analytical and chemometric tools and methodologies, **has been achieved**. In addition, the PhD student managed to draw many interesting conclusions based on a comparison of the results obtained using selected, combined analytical techniques, i.e. direct injection *vs.* SPME; GC-FID *vs.* HS-SPME-GCxGC-TOF/MS *vs.* e-nos using the ultra-fast GC technique; HS-SPME/MS *vs.* HS-SPME-GCxGC-TOF/MS; HS-SPME/MS *vs.* IR/MS. This constitutes a great cognitive value of the research carried out. The doctoral student also showed the ability to use appropriate chemometric tools to classify the tested samples.

The **most valuable** and **most important** achievements of the Ph.D. student described in the four publications presented are:

1. Development of a procedure for the determination of 62 volatile compounds using GC-FID in a system allowing injection into two chromatographic columns of different polarity (CPWax-57CB and DB-624) and independent detection by two independent FID detectors. It turns out that one of the seemingly simpler solutions may be the one that will bring a satisfactory answer. The solution ensured the increased reliability of measurements, and parameters such as linearity, sensitivity, reproducibility and limit of detection ensured the use of the method to control the quality of Baijiu samples. The use of an additional FID detector does not significantly increase the cost of the equipment, while increasing the certainty of the identification of compounds, allowing for the separation of co-eluting compounds and reducing the time by half compared to the analyses using two separate chromatographs. Orthogonal Partial Least Square-Discriminant Analysis (OPLS-DA) did not provide a classification of samples in terms of region, but it was possible to distinguish Baijiu genera in terms of aroma.

2. Comparison of two chromatographic column systems in the HS-SPME-GCxGC-TOF/MS system and demonstration that the system based on the use of a column with stationary non-polar phase (SLB-5) as the first and a column with stationary polar phase (Supelcowax-10) as the second one ensures better separation of volatile compounds, as more analytes are detected when using this configuration. The indicated combination of columns provided better orthogonality and separation power in general, although the opposite configuration provided better separation, e.g. for aldehydes. To classify Baijiu on the basis of the obtained data the chemometric method OPLS-DA was used. Both column configurations provided an excellent discrimination in terms of aroma and geographical origin, however, a larger number of detected compounds supports the use of the system: the non-polar stationary phase-polar stationary phase.

3. Demonstrating that the HS-SPME-GCxGC-TOF/MS technique based on the non-polar stationary phase-polar stationary phase column system provides the best classification of Baijiu samples in terms of aroma type and geographical origin, compared to the developed methods based on GC-FID, SPME-MS and e-nose using the ultra-fast GC technique. This proves the need for a stage of chromatographic separation of volatile

compounds, if the subject of the study is to be the determination of aroma and geographical origin.

4. Obtaining 100 % correctness of the regional (geographical) classification for Baijiu with an intense aroma in the Sichuan, Heilongjiang and Jiangsu regions using HS-SPME-GC and HS-SPME-GCxGC-TOF/MS techniques.
5. Proposing HS-SPME/MS as a technique for testing the so-called "*fingerprint*" of volatile compounds and proving that it can better classify C3 plant-based (potato, rye, wheat) alcoholic beverages than the commonly used isotope ratio mass spectrometry (IRMS) technique. Classification due to botanical origin using HS-SPME-MS was also verified by using an artificial neural network obtaining 100 % compliance.
6. Proposing procedures that after appropriate adaptation can be successfully implemented in industrial laboratories and laboratories of specialized control services to quickly and unambiguously determine the chemical composition during the production process of Baijiu, as well as eliminate adulterations of this product.

The reviewer's task is also to identify shortcomings and ambiguities. In fulfilling this obligation, I state that the text contains a few letter errors (I have counted 3 of them). Below are listed some other minor errors:

• In the abstract in Polish, you mention the limit of quantification of LOD compounds. The acronim LOD refers to the limit of detection.

• In the abstract, you use the term "*rozdział" (division*), and we are dealing with *„rozdzielanie" (separation)*.

• Abstract: What do the terms "C3 and C4 plants" mean? Later, in the text, you give the examples in parentheses, but this should already be placed in the abstract.

• List of abbreviations and acronyms: OPLS-DA – no explanation of the acronym "orthogonal".

• Figure 1.2.: The figure shows 11 aromas, while the caption indicates that there should be 12 of them. Curiosity does not allow me not to ask, what is this missing aroma?

• Figure 3.1. In my opinion, this should include information on the chemometric analyses carried out. This is an extremely important element of the research carried out by the PhD student, and here it was omitted, impoverishing the presentation of the work performed.

• Figures 3.2. and 3.4. The captions under the figures are not very precise. First of all, these are chromatograms obtained from Baijiu samples, using the techniques you are comparing.

I also have some questions concerning the experimental part and the results obtained:

1. Why was Publication 2 (designated JFA2021) comparing two sets of different columns? What I mean is that in the non-polar stationary phase-polar stationary phase system these were SLB-5 and Supelcowax-10 columns, while in the polar stationary phase-non-polar stationary phase system these were Supelcowax10 and Rtx-5 columns. Why didn't you compare the same columns, in different configurations?
2. SPME-GCxGC-TOFMS with non-polar stationary phase was the first one provided better resolution (more chemical compounds were obtained). How can this be explained from the point of view of the separation mechanism? Why did this happen?
3. The Ph.D. student is requested to present the most important personal research achievement of the implemented dissertation.

The above remarks and suggestions do not affect the substantive value of the work. Most of them are debatable. I expect to get an answer to them during the public defense. At the same

time, I believe that in the light of the applicable regulations (the Act of 20 July 2018, the *Law on Higher Education and Science*, revised), the presented dissertation meets the requirements set out in doctoral theses and I apply for admission of **Ms Xi He**, M.Sc. Eng. to pursue further proceedings for a Ph.D. in Agricultural Sciences in the Food and Nutrition Technology discipline.
At the same time, due to the fact that the dissertation under evaluation is innovative (it combines the issues of basic research with practice), and by its interdisciplinarity and innovative subject matter combines many threads in the field of modern analytical chemistry (unique use of combined and conjugated techniques in the determination of volatile components of samples), food and chemometry, as well as the publication of research results in specialized journals from the JCR list with high IF indicators, I move for **awarding it with distinction**.

Toruń 26.05.2023 Bogusław Buszewski