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DOCTORAL THESIS REVIEW

Doctoral dissertation of Xi He, M. Sc. „Gas Chromatography and Hyphenated Techniques in Assessment of Baijiu Spirits Authenticity and Quality”, done under the direction of Prof. dr. Henryk H. Jeleń in the Food Volatilomics and Sensomics Group, Department of Food Technology of Plant Origin, Faculty of Food Science and Nutrition.

In 2023, the spirits market reached a global value of USD 525.31 billion and is expected to grow in the coming years (2023-2027) at an annual rate of 4.56%. In the global ranking, most of the income is generated in China (USD 165.90 billion in 2023). In China's domestic spirits market, Baijiu dominates, accounting for 94 percent of total sales. Most of the Chinese production of this liquor is consumed domestically, while only about two percent was exported. “Baijiu” literally means "white liqueur" in Mandarin. It is a traditional distilled alcoholic beverage that typically contains 35 to 60 percent alcohol. The most common ingredient for making Baijiu is fermented sorghum. However, other grains such as rice and wheat can also be used. Based on the characteristics of the aroma, 12 types of Baijiu can be distinguished, with four of them, i.e. Strong, Sauce, Light and Rise, being the most basic, and all others come from the above-mentioned four. Baijiu is often regarded as China's national drink, and is enjoyed on a variety of occasions, from family gatherings to business banquets (it is projected to consume 5.1 billion liters in 2025).

Such a large market creates the possibility of counterfeiting, which may be related to e.g. using cheaper raw materials, using the non-typical (less cost-intensive) production technology, shortening the maturation period, or the wrong geographical place of origin. Currently, differentiation of Baijiu types based on their aromas is mainly done using sensory evaluation, testing of ingredients used for brewing and analysis of the production process. Expert training usually generates very high costs for enterprises and is difficult to implement under large-scale market control conditions.

Currently, various methods are used to determine the authenticity of alcoholic beverages, based on chromatographic separation, mass spectrometry, spectroscopy and the use of various

sensors. In the case of fermented beverages, including spirits, gas chromatography methods are most often used for this purpose, due to the presence of a large amount of volatile components in the products, whose qualitative and quantitative share may be a kind of "fingerprint" of the analyzed product.

Topics related to the study of various aspects of Baijiu production have been very popular in recent years. The Web of Science Core Collection currently has 713 scientific articles on this topic, of which 644 are from the last 5 years and 642 are original research articles. There are analyzes of microbiological aspects regarding the development of various microorganisms during the Baijiu spontaneous fermentation process and their impact on the product (178), chemical composition studies (213), aspects related to nutritional value (75), or the impact of technology on quality (413). 185 articles concern the use of gas chromatography, while only 7 are related to the detection of adulteration of Baijiu.

Articles published by Ms. Xi He, M.Sc., are part of the above-described trend and provide new methodological and analytical information, linking the chemical composition of Baijiu with their geographical origin, type (related to the production technology), and the raw materials used to obtain the beverages. The presented research results are of great application importance, due to the possibility of using them by producers, distributors, as well as control units to test the authenticity of various batches of Baijiu spirits.

As his scientific achievement, Xi He, M.Sc., presented a series of 4 papers published in 2020-2023 in journals included in the JCR database, i.e. published by John Wiley & Sons, Inc. - Journal of the Institute of Brewing (1 paper), Elsevier publishing house (2 papers) - Food Chemistry and Journal of Chromatography A and ACS publications - Journal of Agricultural and Food Chemistry. The total Impact Factor of articles of doctoral dissertation is 21,512, and the sum of points according to the score of the Ministry of Science and Higher Education, calculated according to the year of publication, is 480. According to Web of Science, the number of citations of these works as of June 16, 2023 is 52. These are team works in which the doctoral student is listed as the first of the Authors, were made in cooperation with other employees of the Department of Food Technology of Plant Origin, Faculty of Food Sciences and Nutrition, Poznań University of Life Sciences. The documentation includes statements of co-authors confirming participation in the creation of these publications, unfortunately there is no percentage share of Xi He, M.Sc., in their formation. According to the information contained in the statements, the PhD Student's role in the preparation of the articles includes co-creating the research concept, obtaining research material, co-developing the chromatographic analytical method along with its verification and validation, performing all GC analyses, interpreting the results and applying appropriate statistical methods, and preparing the publication for printing, as well as correspondence with reviewers, in response to their comments.

The work submitted for evaluation includes 41 pages of typescript. Next, the publications being the subject of the doctoral dissertation and statements of the co-authors of these publications are attached. The list of literature contains 71 items, mostly original and English-language, a significant part of which are works published in international journals from JCR list. Most bibliographic items come from the last 10 years - 51 items, 72%.

The work has been divided into 5 main chapters, it also includes abstracts in Polish and English. The form of the work is clear, and the table of contents placed at the beginning of the study facilitates the reader's orientation and quick access to the issues contained in individual chapters. The proportions of the individual chapters are reasonable.

The Introduction chapter (15 pages) serves as a review of the current literature on the subject of research, it also justifies the validity of the research undertaken and the selection of analytical methods. The author formulated 4 research goals, corresponding to each of the attached publications. In this part, I miss some research hypotheses that the PhD student wanted to prove or reject in the course of her research. The results obtained during the research are briefly discussed on 9 pages. There are selected chromatograms in the text that were to facilitate the visualization of the results, and which were not included in the published articles. The main part of the work ends with 4 general conclusions summarizing the most important achievements obtained in the series of experiments, as well as a short summary.

The dissertation is carefully edited, written in correct language, it indicates a good orientation of the PhD student in the area of the issues raised.

The thesis begins with an Introduction section, introducing the subject of the dissertation. It includes a review of the current state of knowledge on the issues described in the work, including the types of Baijiu, the production technology of the main types (Sauce Baijiu, Strong Baijiu, Light Baijiu), the microorganisms involved in their production, the methods used to determine the authenticity of various spirit drinks (including Baijiu), as well as the analytical and statistical methods used during the experiments.

For this chapter, I have only one comment regarding the types of adulteration of alcoholic beverages mentioned by the author on Page 11. In point 1, the PhD student states that one of the possible adulterations is " The addition of water or other cheaper components without declaration, for instance, adding industrial purpose denatured alcohol or methanol into the product." While the dilution of beverages or the use of cheaper spirits not intended for consumption actually takes place, the addition of methanol, which is highly toxic even at low doses, is extremely rare, and the possible presence of methanol in an alcoholic beverage is not intentional, but rather is the result of the use of low-quality spirit or the use of specific enzymatic treatment during its production.

The aim of the study was to obtain chromatographic methods and/or hyphenated techniques to assess the authenticity and quality of Baijiu alcoholic beverages.

For the experiments, 4 specific objectives were also formulated:

- To identify and quantify more volatile compounds, as well as examine feasibility of accessing the Baijiu geographical origins and aroma types. A simple method was investigated using direct injection onto two different polarity columns simultaneously in the GC-FID system.
- To explore the influence of different column combinations of GC×GC-TOFMS on the determination of Baijiu aroma types and regional origins. The volatile profile analysis from the same set of Baijiu samples by different column setups was carried out.

- To compare HS-SPME-MS with the gas chromatography based electronic nose (GC-E-Nose) in the assessment of authenticity of Baijiu aroma types and regional origin based on the volatile fingerprint, two rapid methods were evaluated.
- To investigate the possibility of volatile fingerprint from HS-SPME-MS in tracing botanical origins of raw spirits, the HS-SPME-MS was collated with IR-MS by analyzing the same set of single ingredient Baijiu and raw spirits.

The chapter "Research Description" contains a brief description of the experiments carried out and the analytical methods used, as well as a discussion of the most important results obtained during the analysis. Descriptions of analytical methods included in the thesis, as well as in the individual publications, have been prepared very clearly and testify to the excellent analytical preparation of the PhD student to conduct this type of research. During experiments, the PhD student used a wide range of analytical and statistical methods such as gas chromatography with a flame ionization detector (GC-FID), solid phase microextraction of compounds from the headspace combined with complete two-dimensional gas chromatography with time-of-flight mass spectrometry (HS-SPME-GC×GC-TOFMS), mass spectrometry without chromatographic separation (HS-SPME-MS), an electronic nose based on ultra-fast gas chromatography (GC-E-Nose) and stable isotope mass spectrometry (IRMS). In addition, for the visualization, interpretation and analysis of the results obtained, statistical analysis methods such as orthogonal partial least squares combined with discriminant analysis (OPLS-DA), multi-block orthogonal component analysis (MOCA), principal component analysis (PCA), and artificial neural networks (ANN) were used.

Chapter 5 presents all four publications included in the doctoral dissertation. In the first of them - "Determination of volatile compounds in Baijiu using simultaneous chromatographic analysis on two columns" published in the Journal of the Institute of Brewing, 126(2), 206-212, a method for the quantification of 62 compounds in Baijiu spirit during simultaneous direct injection on two columns of different polarity (CPWax-57CB and DB-624) was developed. The method was characterized by high linearity ($R^2 \geq 0.999$) and repeatability. The detection limit was <1 mg/L for all compounds. Based on partial least squares orthogonal discriminant analysis, it was not possible to distinguish samples by region of origin, but it was possible to distinguish Baijiu type Strong and type Soy sauce from other samples. I have only one remark for this publication. It is not clear where the authors obtained the information that Baijiu is richer in aromatic compounds than whiskey and brandy, containing about 60 key aromatic compounds, compared to 20 and 30 in the other two spirits, respectively. The article "Mystery behind Chinese liquor fermentation" by Guangyuan Jin, Yang Zhu, Yan Xu from Trends in Food Science & Technology is cited here, which does not contain this kind of information.

In the following publication "Comprehensive two-dimensional gas chromatography–time of flight mass spectrometry (GC× GC-TOFMS) in conventional and reversed column configuration for the investigation of Baijiu aroma types and regional origin" in Journal of Chromatography A, 1636, 461774, comprehensive two-dimensional gas chromatography in a conventional and inverted column system (non-polar - polar and polar - non-polar columns) was used to differentiate the set of 65 Baijiu samples used in the earlier studies in terms of their

type and geographical origin (region). Both column configurations provided excellent Baijiu discrimination of Light, Soy sauce, Feng and Herbal types. A better classification result for the Strong and Jian type Baijiu was found for the conventional column arrangement. Using OPLS-DA, both column configurations were able to distinguish the Strong Baijiu very well depending on the region of origin - Sichuan, Heilongjiang and Jiangsu. I have one question about the publication. In section 2.4, the authors state that "Components that were present in less than 50% of the samples were eliminated...". Wouldn't compounds unique to the beverages allow for greater differentiation between samples in OPLS-DA analyses?

In the third publication "Rapid analysis of Baijiu volatile compounds fingerprint for their aroma and regional origin authenticity assessment.", Food Chemistry, 337, pp.128002, the usefulness of solid phase microextraction-mass spectrometry (SPME-MS) and gas chromatography-based electronic nose (GC-E-Nose) to distinguish Baijiu of different types and geographic origins were compared. A total of 65 Baijiu samples representing 6 aromas and 3 regions (Strong aroma type) were tested. Data were pre-processed for multi-classification models (OPLS-DA) as well as for binary classification ones (PLS-DA). Classification performed using the OPLS-DA method showed that the use of SPME-MS to discriminate between samples was more useful and predictive compared to GC-E-Nose. The total correct classification rate for SPME-MS was 94.44% for aroma and 100% for the region of origin, whereas for the GC-E nose this values was 91.53% and 93.94%, respectively.

The last publication from the series is "Can volatiles fingerprints be an alternative to isotope ratio mass spectrometry in the botanical origin determination of spirits?" Journal of Agricultural and Food Chemistry, 71(5), pp.2637-2643. A quasi-electronic mass spectrometry-based nose using solid phase microextraction to introduce volatiles directly into the mass spectrometer without chromatographic separation (HS-SPME-MS) was used to discriminate between 45 raw spirits produced from C3 (potato, rye, wheat) and C4 crops (maize, sorghum). The samples were also subjected to isotope ratio mass spectrometry (IRMS) which clearly distinguished C3 from C4 samples; however, no clear differentiation was observed among the C3 samples. On the other hand, the HS-SPME-MS method, using the "fingerprints" of unidentified volatile compounds in the form of ions, with a given m/z range and various intensities, provided an excellent classification of samples after data processing using the OPLS-DA method, also verified by an artificial neural network (ANN).

Based on the results presented in all 4 publications, 4 conclusions were drawn, presented in the Conclusions chapter, along with a short summary regarding the possibility of using the analytical methods used to distinguish Baijiu samples depending on their place of origin, type of aroma and plant material used for fermentation.

After reviewing the dissertation submitted, the following questions occurred to me, which I would like to ask PhD student for an answer:

- In the presented studies, the possibility of classifying Baijiu samples depending on the type of aroma, area of production, type of raw material used for production was analyzed, while there

is more factors that may affect the chemical composition of Baijiu. How would a PhD student systematize these factors from most to least significant? Please justify your answer.

- dozens of volatile components were detected during the analysis, could Ms. Xi He group them depending on the origin and mechanism of formation, thus presenting how the main components of the Baijiu aroma are produced?

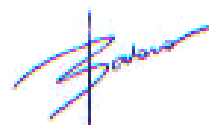
To sum up, I read the work with pleasure, due to many years of professional interest in fermented beverages (including spirits), as well as in the chromatographic methods used for their analysis. I appreciate the diligence of work, aesthetic values, innovativeness of the methods used and the publication of research results in journals from the "top shelf". On the basis of the dissertation submitted, I express the opinion that the PhD student is a person prepared to conduct independent research, has multi-faceted knowledge in the area of the presented subject and is able to clearly present the results of her observations and formulate conclusions.

In the conclusion, I state that the doctoral dissertation of Ms. Xi He, M.Sc., " Gas Chromatography and Hyphenated Techniques in Assessment of Baijiu Spirits Authenticity and Quality", combines scientific and practical aspects, making an important contribution to the field of food science and nutrition. I confirm that the work submitted for review meets the requirements of art. 13 sec. 1 of the Act of March 14, 2003 on academic degrees and academic titles and on degrees and titles in the field of art, and in the provisions amending the Act - Law on Higher Education and Science of July 3, 2018 (Journal of Laws of 2018, item 1669), for candidates applying for a doctoral degree.

For the above reasons, I am asking the Dean and the members of the Scientific Council of the Discipline Food Technology and Nutrition, Poznań University of Life Sciences, to admit Xi He, M.Sc. to the next stages of the doctoral proceedings.

DISTINCTION

At the same time, I would like to propose to the Honorable Scientific Council of the Discipline of Food Technology and Nutrition, Poznań University of Life Sciences, awarding doctoral thesis of Ms. Xi He, M.Sc., "Gas Chromatography and Hyphenated Techniques in Assessment of Baijiu Spirits Authenticity and Quality". I justify my proposal with the enormity of work that the PhD student had to put into such a comprehensive analysis of the issue, an innovative approach to the analysis of adulteration of spirits, as well as high reliability in the presentation of the results. It is also worth emphasizing the use of very advanced analytical techniques based on chromatography and/or spectrometry, such as HS-SPME-GC×GC-TOFMS, HS-SPME-MS, GC-E-Nose and IRMS and statistical ones, such as OPLS-DA, MOCA or ANN, as well as publishing research results in journals from the "top shelf ".



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