Current Clinical and Medical Education

Received 19 Mar 2024 | Revised 20 Mar 2024 | Accepted 24 Apl 2024 | Published Online 2 May 2024

vision publisher
Medical Journals

Published By: Vision Publisher
ISSN 2942-0792

CCME 02 (5), 49-56

Original Article

# Nuclear Magnetic Resonance (NMR) Spectroscopy for the Examination of Food Composition, Fermentation, and Metabolic Alterations

Abbas Mohammed Abbas Ali Alghaziy[1], Ayat Khayr Allah Hady Almaealij Aleinziu[2], Noor Al-Huda Hasan Hadi Asfoor[3], Zeyad Kazem Jabr Sagit[4], Aqeel Faisal Waheed[5], Hussein Abd AL-Meer Salal[6], Ahmed Hamza Oribi Al-Amiri[7]

[1]University of Kufa, College of Science, Iraq

[2]Dhi Qar University, College of Psychology, Iraq

[3,4,5,6,7]Hilla University College, Iraq

**Abstract:** We detail the three main types of nuclear magnetic resonance (NMR) methods used to analyze foodstuffs and provide examples of each. Two approaches can be taken here: one involves studying the food of interest using various complementary NMR methodologies to get a complete picture of its structure and composition; the other involves using the food's specific problem (food fraud, safety, traceability, geographical and botanical origin, farming methods, food processing, maturation and ageing, etc.) to determine which NMR methodology is most suited; and lastly, one can start with a single NMR methodology and develop a wide range of applications to address common food-related challenges and other food-related aspects using that methodology. One of the most potent methods for studying chemical reaction processes, analyzing sample components, and determining compound structures is nuclear magnetic resonance (NMR) spectroscopy. Initial applications of nuclear magnetic resonance (NMR) in the investigation of food moisture were as a quick and noninvasive analytical tool. Oil, protein, and carbohydrate analysis can reveal a food's structural composition. More and more, food scientists are using NMR in conjunction with multivariate statistical data. Despite its limitations in sensitivity and technical understanding, nuclear magnetic resonance (NMR) remains a powerful tool with promising future applications in food research. In this overview, we will look at how nuclear magnetic resonance (NMR) can be used to analyze various foods, such as oils, red wines, honey, milk, fruits, and vegetables. NMR has many uses: it can detect chemicals rapidly, determine where species came from, evaluate food quality, expose fraud, and estimate how long food will stay fresh.

**Keywords: NMR; food science; multivariate statistical analysis; food quality.**

**Corresponding Author:** Abbas Mohammed Abbas Ali Alghaziy†, University of Kufa, College of Science, Iraq

**Introduction:** Many different areas fall under the umbrella of food science. When studying food, its degradation, processing principles, and transformations, it blends fundamental subject theories from fields such applied biology, chemistry, physics, medicine, and engineering [1]. Metabolic reactions in plant development and food processing create extremely complex matrices with thousands of components, which are what we call foods.

A number of factors, including variations in farming techniques, feeding practices, and processing processes, as well as environmental and climatic factors, affect the genetic and geographical components of food. Minerals, water, phenols, proteins, carbohydrates, lipids, and amino acids make up the bulk of it. Chemical reactions can occur during storage, transit, processing, and the addition of flavoring agents to alter the composition of food, which in turn affects its nutrition, taste, color, and quality [2].

People have started to pay more attention to the nutrition and quality of food—its flavor and taste, shelf life, type, origin, year, and so on—because it is the fundamental physiological and biochemical energy source for humans. Consequently, picking the right technologies to test food composition, track changes in food composition after processing and transportation, and forecast food shelf life is becoming more and more critical in the food industry.

One spectroscopic tool for analyzing chemical composition and physical characteristics is nuclear magnetic resonance (NMR). Nuclear magnetic resonance (NMR) found its first usage in physics before finding widespread use in chemistry, biology, medicine, and other disciplines. As far back as 1957, when low-field NMR was utilized to quantify food water content, NMR found a home in the food science area. Before the 1980s, nuclear magnetic resonance (NMR) was not commonly utilized for food analysis because of its immature technical theory, low sensitivity, and expensive equipment costs [3, 4]. Oil, vegetables, wine, and dairy products are just a few examples of the many foods that have benefited from nuclear magnetic resonance (NMR) analysis thanks to developments in probe technology, superconducting magnets, Fourier transform spectroscopy, and high-throughput technology.

The primary application of high-resolution NMR is to acquire frequency domain spectra for the purpose of documenting chemical structures. Low-field nuclear magnetic resonance (LF-NMR) is a method that measures the diffusion coefficient D and relaxation time T1/T2 to reflect the mobility characteristics of individual protons in a sample, and it gives information on the chemical and physical surroundings of the sample. MRI relies on shifts in relaxation duration, as well as changes in the distribution and mobility of water in meals.

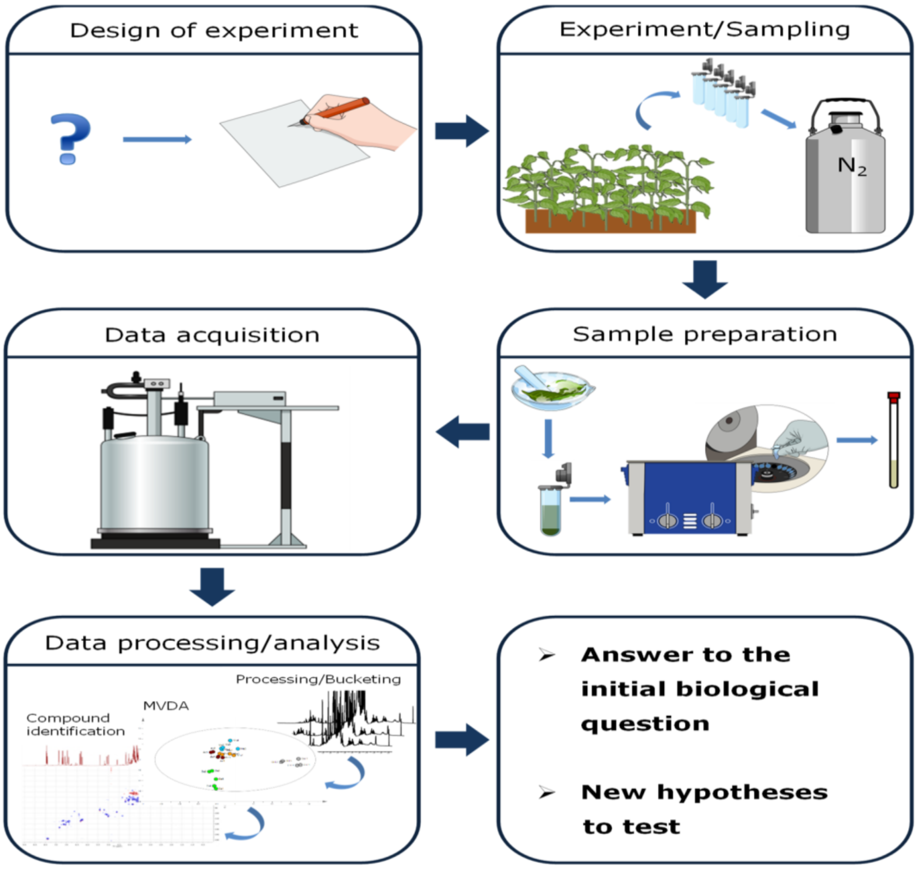

**Figure 1.** Biologically and statistically significant results require a well-designed experimental method, which is what an NMR-based metabolomics pipeline aims to provide. All the steps from sampling to data gathering and analysis are covered in this (Figure made with the graph platform in mind).

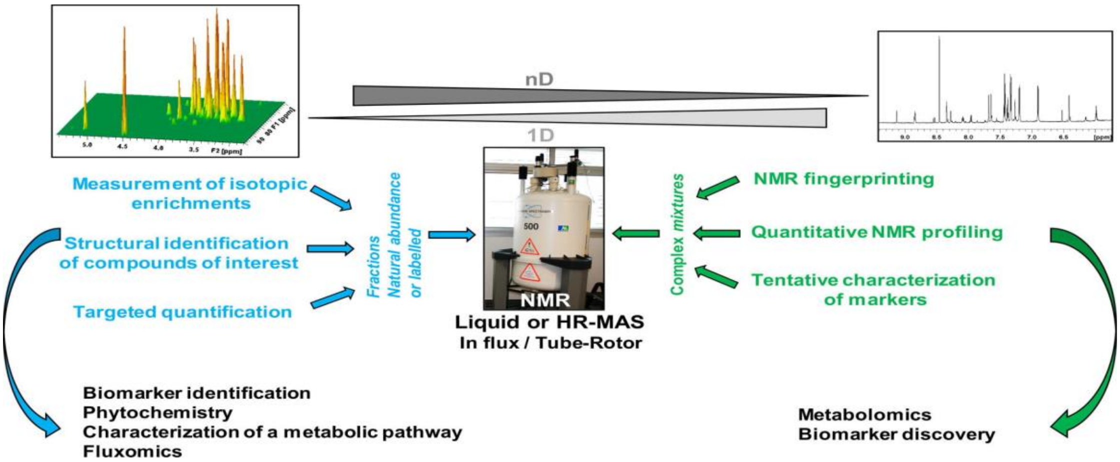

**Figure 2. Nuclear magnetic resonance (NMR) analysis of plant metabolism.**

Nuclear magnetic resonance spectroscopy has made numerous varied contributions to our understanding of primary and specialized metabolism in plants. Despite mass spectrometry's (MS) superior sensitivity, nuclear magnetic resonance (NMR) has many benefits, including being quantitative, non-destructive, non-specific, and reproducible. Research on subcellular pH in tomato fruit using 13C NMR [5], carbon deprivation in cells or glyphosate herbicide sequestration in vacuole using 31P, and other in vivo NMR experiments are not covered in this review but have added to our knowledge of metabolism. We will not go into depth about the use of plant NMR imaging, [6] here, despite the fact that it is a powerful tool for seeing plant metabolites and internal structures and could provide important information about plant physiology and development. Instead, we'll highlight the significance of liquid-state and HR-MAS (High Resolution Magic Angle Spinning) nuclear magnetic resonance spectroscopy in the field of plant metabolic research. These two methods have a lot of promise for green biotechnology in the areas of biofuels and biomaterials, but they also have a lot of promise for characterizing food and other samples generated from plants [7–10]. But these uses are beyond the purview of this evaluation at the moment.

For metabolomics and other areas involving plant metabolism and organic chemical composition, NMR can be employed either alone or in conjunction with other analytical techniques like MS [11–13]. In fact, nuclear magnetic resonance (NMR) and nuclear magnetic resonance (MS) work well together; whilst MS is primarily useful for its great sensitivity, NMR has other benefits, such as a high degree of repeatability and access to clear structural information. This article summarizes the most up-to-date research on plant metabolism using one-dimensional and multi-dimensional nuclear magnetic resonance (NMR) techniques. In particular, it will demonstrate how nuclear magnetic resonance (NMR) has been a game-changer for targeted phytochemistry by allowing for the identification and quantification of compounds, as well as for the worldwide characterization of sample composition in metabolomic research. For this reason, nuclear magnetic resonance (NMR) methods are frequently employed to offer chemical snapshots of plant samples. Despite this, metabolic networks are in a constant state of flux, and NMR methods have been employed in certain specific instances to quantify metabolic fluxes following stable isotope labeling for decades. Accordingly, both 1D and 3D nuclear magnetic resonance (NMR) methods for fluxomics are detailed in the review. The primary audience consists of nuclear magnetic resonance (NMR) experts who are keen to delve deeper into the applications and capabilities of their preferred method in the realm of plant sciences. So, to help you out, we've provided a paragraph outlining the exact measures to take when getting your samples ready. Furthermore, a particular section is devoted to data processing and data exchange, since these factors greatly impact the quality of NMR metabolic research.

## Time spent fermenting

While grapes may supply some of the chemical building blocks for wine, the majority of the chemicals are actually made by yeast and lactic acid bacteria when they undergo metabolic activities during the winemaking process. Using nuclear magnetic resonance (NMR), we tracked the fermentation of histidine and malic acid in wine as it transformed into alcohol. Malic acid lactic acid fermentation turned histamine into histamine, and alcohol fermentation turned histidine into histamine alcohol [14]. On the other hand, lactic acid bacteria can be used to prevent histamine generation during the malic acid lactic acid fermentation process. Using 1H NMR spectroscopy, we can compare the metabolites produced by commercially available yeast starters with those of a few carefully chosen Saccharomyces cerevisiae starters that we create ourselves, allowing us to clearly identify wines that have been fermented with distinct yeast sources. The impact of non-yeast microorganisms and various techniques of managing malolactic fermentation on the quality of Barbera red wine during alcohol fermentation. The primary metabolite alterations during AF and MLF were identified using 1H NMR spectroscopy in conjunction with multivariate statistical analysis [15].

Wines made with a blend of nonyeast bacteria and malolactic acid fermenting in the same tank might have a fuller body and more complex flavor profile. Organic and biodynamic wine identification was the subject of another investigation that employed 1H NMR and metabonomics. Amino acids and organic acids were associated with the chemical shift zone between 0.8 and 4.0 ppm, whereas phenols were located in the specific spectral band between 5.5 and 8.5 ppm. Tyrosine and resveratrol were abundant in the organic brewing plan, but trans-caffeic acid was scarce. The results demonstrated that the metabolic properties of wine are most affected by the production year and the winemaking strategy.

The polyphenols in wine are responsible for their distinct flavors and hues. Polyphenols undergo gradual condensation and oxidation processes as wine ages, resulting in the formation of oligomeric and polymeric derivatives[16, 17]. The precipitates collected from 2012 and 2013 Bordeaux wines were quantitatively analyzed using solid-state NMR. The samples were subjected to temperatures of -4 °C or 4 °C for 2-6 days, and the sediment's evolution over the course of a year was tracked. It rained more heavily in 2012 than in 2013 while temperatures were low. In addition to organic acids and free amino acids, the sediment primarily included potassium tartrate, polyphenols, a trace quantity of polysaccharides, and tartaric acid, which is crucial for wine stability.

This would mean that the amount of rain that falls has more to do with the year, the weather, and the type of grape than with how old the grapes are. Using 1H NMR spectra in conjunction with statistical analysis, ten distinct wines were able to be differentiated between the two maturing stages after being bottled for three months and kept at 12 °C for forty-eight months. Modest alterations in metabolites may also occur as wine ages. As people get older, they tend to have lower levels of organic acids such tartaric acid, lactic acid, and succinic acid, and higher levels of ethyl acetate and milk [18]. It is possible that the hydrolysis of tannins is responsible for the decrease in catechin and epicatechin concentration in polyphenols and the increase in gallic acid in nearly all red wines. Although NMR metabolic analysis has a lower sensitivity than other analytical methods, it is useful for providing a full description of wine's evolution.

## Activation of water

Measuring water activity is essential in food, particularly after drying, since it dictates the product's stability. Water content has an effect on growth and metabolism, and fruits and vegetables are rich in it [19, 20]. The microscopic growth, storage, and decay of fruits and vegetables can be better understood by measuring the dynamic changes in water. To determine the level of moisture in vegetables, Lv recently developed a device that combines microwave vacuum drying (MVD) with low field nuclear magnetic resonance (NMR). To represent the water condition, the signal amplitude T2 was utilized. While MVD resulted in the loss of many types of water, the most common of these was free water, which varied among vegetables. A linear fitting model can only utilize A2 to predict when drying will be complete, even if there is a strong correlation between the water content and the signal amplitudes of bonded water, fixed water, free water, and entire water (A2) at various stages of drying.

The water states of several fruits and vegetables were tracked using LF-NMR spectroscopy while they were dried. A leftward shift of the T2 peak and a decrease in water activity occurred with drying [21]. It is possible that the sugar's change in condition during drying causes a decrease in shrinkage, since the T2 values of sugar-treated and plain dried samples were comparable and exhibited a downward trend. On the other hand, tissue damage and a longer T2 relaxation period are observed in samples treated with salt. The samples treated with salt or sugar had a similar effect on water activity, but the effect on T2 relaxation time was opposite. Both fresh and dried apples were tested for their polyphenol content and antioxidant capacity by Francini [22]. The six varieties of fresh apples varied in the amount of total polyphenols they contained. Even though the capacity to absorb oxygen radicals dropped following dehydration, the antioxidant activity remained strong. With a correlation coefficient of 0.842 ($P < 0.001$), the data demonstrated that the amount of phenol had a significant impact on the antioxidant activity.

Bruker and SGF International collaborated to create spin-generated fingerprint profiling (SGF-Profile) as a means of analyzing fruit juice. At present, the database contains the spectra of over three thousand reference juices, encompassing over thirty distinct fruit varieties from over fifty different nations. A total of 29 components in apple juice were quantitatively examined using the SGF-PROFILE system's automated execution data analysis and report. These compounds included sugars, amino acids, organic acids, acetyl groups, arbutin, benzaldehyde, hydroxymethyl furfural, acetaldehyde, methanol, and ethanol. It may also identify different types of fruit and products, whether they have added sugar or acid, where they came from, and whether they are mixed fruits, depending on the model employed.

## Produce and fresh produce

Fruits and vegetables have made extensive use of nuclear magnetic resonance spectroscopy for characterization. There are certain components of all fruits and vegetables that are associated with their biological activity, scent, flavor, and nutritional value [23]. Pomegranates from several parts of Iran were studied using 1H NMR and 2D NMR spectroscopy in order to refine the ecotype of pomegranates. Glucoside, glucose, and sucrose were the primary components of the pomegranate juice spectrum.

It is possible to utilize citric acid and malic acid, the two primary organic acids, to distinguish between various ecosystems, which may be associated with variations in weather and terrain. Despite noticeable differences in total phenol concentration, pomegranate juices from Pakistan, Fordoss, and Kashma have comparable amino acid and sugar spectra. It is possible to differentiate the three production regions using phenolic compounds [24]. The primary metabolites in cellular processes, amino acids also provide light on the botanical ancestry of fruits. Botoran's group isolated ten distinct amino acids, with the exception of glutamic acid, that varied significantly between juice varietals. Stoichiometric approaches (PCA, LDA) can accurately categorize plant juices according to their amino acid profiles.We used nuclear magnetic resonance to examine the Piemonte apple metabolite spectrum. The proportions of glucose and sucrose can vary greatly from one apple variety to another, while fructose appears to be ubiquitous. The high chlorogenic acid level is a hallmark of most Piemonte cultivars. The most common type of fatty acid is di unsaturated, with saturated fatty acids coming in second. Citric acid, rhamnol, and phloridin/phloridin are three of the most useful components for apple variety identification [25].

Fruit metabolite variations during ripening can also be detected using NMR. It was utilized to describe and clarify the variations in key metabolites throughout the four phases of fruit ripening. Metabolism of amino acids, reduction of most organic acids utilized as respiratory substrates to generate ATP, and possible degradation of starch or sucrose to fructose and glucose are all processes that occur during maturation. Black raspberry's biological action is associated with phenolic substances including epicatechin and vanillin [26].

If you are looking for information about the medicinal properties of black raspberry, NMR is a great place to start. At five distinct stages of banana maturity, the changes in metabolites were shown by 1D and 2D NMR spectra in conjunction with principal component analysis (PCA). Metabolites mostly consisted of sugar, amino acids, organic acids, and fatty acids. Banana quality was impacted by the five steps of carbohydrate and amino acid metabolism that occurred throughout aging. The quality was obviously higher in stages three and four. Another indication of banana aging was the conversion of dopamine to salbutamol in the fifth stage. One way to objectively measure whether fruit is ripe or starting to mature is with NMR spectrum data.

## $^1$H NMR and PCA-based analysis revealed variety dependent changes in phenolic contents of apple fruit after drying

Researchers have used 1H NMR to examine the polyphenolic profiles, antioxidant capabilities, and total polyphenol content of both fresh and dried apples. Using an air-drying system set at 45°C for 19 hours, six old and underappreciated apple varieties—Mantovana, Mora, Nesta, Cipolla, Ruggina, and Sassola—along with a commercial apple, Golden Delicious, were dried. Some apple types maintained a higher antioxidant capability than Golden Delicious, even when some of its polyphenol components were lost after drying [26]. For older, less-used cultivars, this finding is crucial since their unattractive appearance prevents them from being consumed in great quantities as a fresh product. To determine the nutritional worth of the many apple cultivars studied, we used a combination of quantitative nuclear magnetic resonance spectroscopy and principal component analysis to identify and quantify numerous polyphenols, including epicathechin, chlorogenic acid, and catechin.

### Metabolomic analyses of banana during postharvest senescence by $^{1}$H-high resolution-NMR

People all throughout the globe love bananas, a tropical fruit. Organoleptic qualities and nutritional value are greatly influenced by its chemical composition. Employing nuclear magnetic resonance spectroscopy, this study examined the metabolite alterations that occur during postharvest senescence. Secondary and primary metabolites, such as organic acids, amino acids, carbs, and phenolics, were uncovered by 1D and 2D NMR spectroscopic data from banana fruit [27, 28]. While all five senescence stages of bananas displayed comparable chemical profiles, the amounts of the various components varied greatly. Postharvest senescence in bananas was caused by valine, alanine, aspartic acid, choline, acetate, glucose, gallic acid, malic acid, and dopamine, among other metabolites. Stage V saw the presence of ethanol as a result of glucose conversion. A byproduct of the dopamine conversion was salsolinol. This was a telltale sign of banana ripening after harvest.

### $^{1}$H NMR-based metabolomics for the discrimination of celery (Apium graveolens L. var. dulce) from different geographical origins

The nutritious richness and presence of bioactive metabolites in celery (Apium graveolens L. var dulce) make it a popular vegetable for cultivation and consumption. A combination of 1H NMR spectroscopy and multivariate statistical analyses was employed to differentiate between geographically distinct celery stem and leaf samples in this investigation [29]. The disparities among celery extracts from three different countries—China, Taiwan, and Australia—were examined using principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA). Mannitol was identified as a strong discriminating metabolite [30–32], and it was shown that organic acids, sugars, and amino acids all play a role in the distinction between origins. Metabolite biomarkers that could be used for regional origin differentiation can be identified using NMR-based metabolomics, which has shown to be an excellent method for creating trustworthy metabolomic fingerprints and profiles.

## CONCLUSION

Chemical composition, molecular structure, and compound changes can be elucidated with the use of nuclear magnetic resonance spectroscopy, a crucial analytical tool. A more profound appreciation for food is being fostered. Food authenticity, safety, quality, and provenance are all areas it can be applied to. Nuclear magnetic resonance (NMR) is a quick and easy analytical technique that may be used on any type of food due to its accuracy and repeatability.

Data analysis using nuclear magnetic resonance (NMR) technology is intricate and requires specialists. The use of nuclear magnetic resonance (NMR) for food detection is restricted due to its limited sensitivity in comparison to other technologies. Spectral resolution is affected by the instrument's sensitivity, the strength of the magnetic field, the experiment's kind, the sample's parameters, and the type of probe used. Data analysis and applications can greatly benefit from NMR spectra databases of certain foods. The future of nuclear magnetic resonance (NMR) technology in the food science sector is bright, especially as the technology continues to advance.

## REFERENCES

1. Alberdi-Cedeño, J., Ibargoitia, M. L., & Guillén, M. D. (2019). Monitoring of minor compounds in corn oil oxidation by direct immersion-solid phase microextraction-gas chromatography/ mass spectrometry. Food Chemistry, 290, 286-294.

2. Buckland, G., & Gonzalez, C. A. (2015). The role of olive oil in disease prevention: a focus on the recent epidemiological evidence from cohort studies and dietary intervention trials. British Journal of Nutrition, 113(Suppl. 2), S94-S101. http://dx.doi.org/10.1017/ S0007114514003936. PMid:26148926.
3. Cai, S., Zhang, Y., Xia, F., Shen, G., & Feng, J. (2019). An expert system based on 1H NMR spectroscopy for quality evaluation and adulteration identification of edible oils. Journal of Food Composition and Analysis, 84(2), 103316
4. Alonso-Salces, R. M., Holland, M. V., & Guillou, C. (2011). 1H-NMR fingerprinting to evaluate the stability of olive oil. Food Control, 22(12), 2041-2046.
5. Curti, E., Pardu, A., Vigo, S., Sanna, R., & Anedda, R. (2019). Non- invasive monitoring of curd syneresis upon renneting of raw and heat-treated cow's and goat's milk. International Dairy Journal, 90, 95-97.
6. D'Imperio, M., Gobbino, M., Picanza, A., Costanzo, S., Corte, A. D., & Mannina, L. (2010). Influence of harvest method and period on olive oil composition: an NMR and statistical study. Journal of Agricultural and Food Chemistry, 58(20), 11043-11051.
7. Dais, P., & Hatzakis, E. (2013). Quality assessment and authentication of virgin olive oil by NMR spectroscopy: a critical review. Analytica Chimica Acta, 765, 1-27.
8. Amargianitaki, M., & Spyros, A. (2017). NMR-based metabolomics in wine quality control and authentication. Chemical and Biological Technologies in Agriculture, 4(1), 9.
9. Gougeon, L., Costa, G., Richard, T., & Guyon, F. (2019b). Wine authenticity by quantitative 1H NMR versus multitechnique analysis: a case study. Food Analytical Methods, 12(4), 956-965. http://dx.doi.= org/10.1007/ s12161-018-01425-z.
10. Hills, B. P., Wright, K. M., & Gillies, D. G. (2005). A low-field, low- cost Halbach magnet array for open-access NMR. Journal of Magnetic Resonance, 175(2), 336-339.
11. McDowell, D., Defernez, M., Kemsley, E. K., Elliott, C. T., & Koidis, A. (2019). Low vs high field 1H NMR spectroscopy for the detection of adulteration of cold pressed rapeseed oil with refined oils. LWT, 111, 490-499.
12. Schievano, E., Finotello, C., Uddin, J., Mammi, S., & Piana, L. (2016). Objective definition of monofloral and polyfloral honeys based on NMR metabolomic profiling. Journal of Agricultural and Food Chemistry, 64(18), 3645-3652.
13. Ball, D. W. (2007). The chemical composition of honey. Journal of Chemical Education, 84(10), 1643.
14. Laghi, L., Versari, A., Marcolini, E., & Parpinello, G. P. (2014). Metabonomic investigation by 1H-NMR to discriminate between red wines from organic and biodynamic grapes. Food and Nutrition Sciences, 5(1), 52-59.
15. Balthazar, C. F., Guimarães, J. T., Rocha, R. S., Pimentel, T. C., (2021). Nuclear magnetic resonance as an analytical tool for monitoring the quality and authenticity of dairy foods. Trends in Food Science & Technology, 108, 84-91.
16. Kaškonienė, V., & Venskutonis, P. R. (2010). Floral markers in honey of various botanical and geographic origins: a review. Comprehensive Reviews in Food Science and Food Safety, 9(6), 620-634.
17. Nardi, T., Panero, L., Petrozziello, M., Guaita, M., Tsolakis, C., Cassino, C., Vagnoli, P., & Bosso, A. (2019). Managing wine quality using Torulaspora delbrueckii and Oenococcus oeni starters in mixed fermentations of a red Barbera wine. European Food Research and Technology, 245(2), 293-307.
18. Schievano, E., Stocchero, M., Zuccato, V., Conti, I., & Piana, L. (2019). NMR assessment of European acacia honey origin and composition of EU-blend based on geographical floral markers. Food Chemistry, 288, 96-101.

19. Zheng, X., Zhao, Y., Wu, H., Dong, J., & Feng, J. (2016). Origin identification and qantitative analysis of honeys by nuclear magnetic resonance and chemometric techniques. Food Analytical Methods, 9(6), 1470-1479

20. Tanno, R., Kato, S., Shimizu, N., Ito, J., Sato, S., Ogura, Y., Sakaino, M., Sano, T., Eitsuka, T., Kuwahara, S., Miyazawa, T., & Nakagawa, K. (2020). Analysis of oxidation products of α-tocopherol in extra virgin olive oil using liquid chromatography-tandem mass spectrometry. Food Chemistry, 306, 125582.

21. Frédérich, M.; Wauters, J.-N.; Tits, M.; Jason, C.; de Tullio, P.; Van der Heyden, Y.; Fan, G.; Angenot, L. Quality Assessment of Polygonum cuspidatum and Polygonum multiflorum by $^1$H NMR Metabolite Fingerprinting and Profiling Analysis. Planta Med. 2010, 77, 81–86.

22. Cardoso, S.; Maraschin, M.; Peruch, L.A.M.; Rocha, M.; Pereira, A. A Chemometrics Approach for Nuclear Magnetic Resonance Data to Characterize the Partial Metabolome Banana Peels from Southern Brazil. J. Integr. Bioinf. 2017, 14.

23. Pariyani, R.; Ismail, I.S.; Ahmad Azam, A.; Abas, F.; Shaari, K. Identification of the compositional changes in Orthosiphon stamineus leaves triggered by different drying techniques using $^1$H NMR metabolomics. J. Sci. Food Agric. 2017, 97, 4169–4179.

24. de Falco, B.; Incerti, G.; Bochicchio, R.; Phillips, T.D.; Amato, M.; Lanzotti, V. Metabolomic analysis of Salvia hispanica seeds using NMR spectroscopy and multivariate data analysis. Ind. Crop. Prod. 2017, 99, 86–96.

25. Bhatia, A.; Bharti, S.K.; Tewari, S.K.; Sidhu, O.P.; Roy, R. Metabolic profiling for studying chemotype variations in Withania somnifera (L.) Dunal fruits using GC–MS and NMR spectroscopy. Phytochemistry 2013, 93, 105–115.

26. Mishra, S.; Gogna, N.; Dorai, K. NMR-based investigation of the altered metabolic response of Bougainvillea spectabilis leaves exposed to air pollution stress during the circadian cycle. Environ. Exp. Bot. 2019, 164, 58–70. [Google Scholar] [CrossRef

27. Fan, G.; Tao, L.-h.; Yue, Q.-h.; Kuang, T.-t.; Tang, C.; Yang, Y.-d.; Luo, W.-z.; Zhou, X.-d.; Zhang, Y. Metabolic Discrimination of Rhizoma Coptidis from Different Species Using $^1$H NMR Spectroscopy and Principal Component Analysis. Planta Med. 2012, 78, 641–648.

28. Verma, A.; Parihar, R.; Baishya, B. Identification of metabolites in coriander seeds (Coriandrum Sativum L.) aided by ultrahigh resolution total correlation NMR spectroscopy. Magn. Reson. Chem. 2019, 57, 304–316.

29. Mediani, A.; Abas, F.; Khatib, A.; Maulidiani, H.; Shaari, K.; Choi, Y.H.; Lajis, N.H. $^1$H-NMR-based metabolomics approach to understanding the drying effects on the phytochemicals in Cosmos caudatus. Food Res. Int. 2012, 49, 763–770.

30. Mandrone, M.; Scognamiglio, M.; Fiorentino, A.; Sanna, C.; Cornioli, L.; Antognoni, F.; Bonvicini, F.; Poli, F. Phytochemical profile and α-glucosidase inhibitory activity of Sardinian Hypericum scruglii and Hypericum Hircinum. Fitoterapia 2017, 120, 184–193.

31. Mannina, L.; Sobolev, A.P.; Capitani, D. Applications of NMR metabolomics to the study of foodstuffs: Truffle, kiwifruit, lettuce, and sea bass. Electrophoresis 2012, 33, 2290–2313.

32. Wang, Y.; Tang, H.; Nicholson, J.; Hylands, P.; Sampson, J.; Whitcombe, I.; Stewart, C.; Caiger, S.; Oru, I.; Holmes, E. Metabolomic Strategy for the Classification and Quality Control of Phytomedicine: A Case Study of Chamomile Flower (Matricaria recutita L.). Planta Med. 2004, 70, 250–255. [Google Scholar]