Open access • Journal Article • DOI:10.1016/J.TIFS.2019.02.001

A review on nuclear overhauser enhancement (NOE) and rotating-frame overhauser effect (ROE) NMR techniques in food science: Basic principles and applications

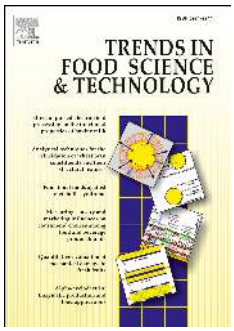
Ali Sedaghat Doost, Marzieh Akbari, Christian V. Stevens, Arima Diah Setiowati ...+1 more authors

Institutions: Ghent University, University of Isfahan

Published on: 01 Apr 2019 - Trends in Food Science and Technology (Elsevier)

Topics: Nuclear Overhauser effect and Two-dimensional nuclear magnetic resonance spectroscopy

Accepted Manuscript



A review on nuclear Overhauser enhancement (NOE) and rotating-frame Overhauser effect (ROE) NMR techniques in food science: basic principles and applications

Ali Sedaghat Doost, Marzieh Akbari, Christian V. Stevens, Arima Diah Setiowati, Paul Van der Meeren

PII: S0924-2244(18)30674-5

DOI: https://doi.org/10.1016/j.tifs.2019.02.001

Reference: TIFS 2407

To appear in: *Trends in Food Science & Technology*

Received Date: 27 September 2018

Revised Date: 13 January 2019

Accepted Date: 1 February 2019

Please cite this article as: Doost, A.S., Akbari, M., Stevens, C.V., Setiowati, A.D., Van der Meeren, P., A review on nuclear Overhauser enhancement (NOE) and rotating-frame Overhauser effect (ROE) NMR techniques in food science: basic principles and applications, *Trends in Food Science & Technology*, https://doi.org/10.1016/j.tifs.2019.02.001.

Background

The characterization of the original chemical structure and induced changes of micro- and macro-molecules using analytical techniques with concise and detailed outcomes is potentially one of the major challenges for food scientists. To this end, the non-invasive nuclear magnetic resonance (NMR) technique can play a significant role through employment of different NMR methods. The Nuclear Overhauser effect (NOE) and rotating-frame Overhauser effect (ROE) techniques are powerful NMR methods that have attracted great interest because they provide precise information about the three dimensional spatial structure of the molecules, as well as about possible chemical reactions and interactions.

Scope and approach

In this article, we reviewed the basic principles as well as applications of two NMR techniques: Nuclear Overhauser effect spectroscopy (NOESY) and rotating-frame Overhauser effect spectroscopy (ROESY). Hereby, we focused mainly on the applications and importance of these techniques in food science research. Both the structural (configuration and conformation) changes and the complexes formed by interacting compounds could be better studied using these techniques.

Key findings and conclusions

The inter- and intra-molecular interactions within food-based ingredient mixtures, as well as configurational and conformational analyses can be more efficiently studied with the aid of NOESY and ROESY. These methods as complementary analysis tools can be exploited for the straightforward elucidation of the spatial proximity of either novel, native or modified compounds. In the future, these techniques may be helpful to better understand the interaction between polymers, such as protein-polysaccharide interactions.

A review on nuclear Overhauser enhancement (NOE) and rotating-frame Overhauser effect (ROE) NMR techniques in food science: basic principles and applications

Ali Sedaghat Doost[1], Marzieh Akbari[2], Christian V. Stevens[3], Arima Diah Setiowati[1], Paul Van der Meeren[1]

[1]Particle and Interfacial Technology Group (PaInT), Department of Green Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, Gent 9000, Belgium

[2]Central Laboratory, Department of Analytical Chemistry, University of Isfahan, Isfahan, 81746-73441, Iran

[3]SynBioC Research Group, Department of Green Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, Gent 9000, Belgium

Background

The characterization of the original chemical structure and induced changes of micro- and macro-molecules using analytical techniques with concise and detailed outcomes is potentially one of the major challenges for food scientists. To this end, the non-invasive nuclear magnetic resonance (NMR) technique can play a significant role through employment of different NMR methods. The Nuclear Overhauser effect (NOE) and rotating-frame Overhauser effect (ROE) techniques are powerful NMR methods that have attracted great interest because they provide precise information about the three dimensional spatial structure of the molecules, as well as about possible chemical reactions and interactions.

Scope and approach

In this article, we reviewed the basic principles as well as applications of two NMR techniques: Nuclear Overhauser effect spectroscopy (NOESY) and rotating-frame Overhauser effect spectroscopy (ROESY). Hereby, we focused mainly on the applications and importance of these techniques in food science research. Both the structural (configuration and conformation) changes and the complexes formed by interacting compounds could be better studied using these techniques.

Key findings and conclusions

The inter- and intra-molecular interactions within food-based ingredient mixtures, as well as configurational and conformational analyses can be more efficiently studied with the aid

of NOESY and ROESY. These methods as complementary analysis tools can be exploited for the straightforward elucidation of the spatial proximity of either novel, native or modified compounds. In the future, these techniques may be helpful to better understand the interaction between polymers, such as protein-polysaccharide interactions.

Keywords

Nuclear magnetic resonance, Nuclear Overhauser enhancement, rotating-frame Overhauser effect, stereochemical structure, complex inclusion

1. Introduction

The nuclear magnetic resonance technique, known as NMR spectroscopy, has been widely utilized for more than 70 years. The early observation of NMR in solids and liquids goes back to the 1940s when Isidor Rabi measured the magnetic properties of some nuclei awarding the Nobel Prize. By now, the NMR technique is a frequently used analytical method in basic and advanced research in different areas, mainly organic chemistry (Kumar and Rani Grace, 2017). There are two types of NMR based on the resolution of the obtained spectra: low and high resolution NMR. High resolution NMR spectra provide more concise information about individual groups within one single molecule whereas these individual contributions become unclear in low resolution NMR. For the first time, the proton magnetic resonance spectrum was recorded at 30 MHz and explained by Arnold et al. (1951) as a direct relation between the chemical shifts and the chemical structure of ethanol. Since the 1950s, nuclear Overhauser enhancement (NOE) has become one of the powerful and common tools in structural elucidation; the Overhauser effect of the nuclear resonance in metallic lithium was initially observed through saturation of the electron spin resonance (Vögeli, 2014, Carver and Slichter, 1953). The rotating-frame Overhauser effect (ROE), which is basically similar to NOE, could be a potential alternative for NOE where the NOE peaks become tremendously weak in the case of mid sized molecules with molecular masses of about 1000 - 2000 Da. However, this phenomenon of decreasing NOEs is dependent on the solution environment and the spectrometer frequency (Claridge, 2016b). The rotating-frame Overhauser effect spectroscopy (ROESY) method was firstly proposed by Bothner-By et al. (1984) to overcome the abovementioned problems. These two NMR techniques are widely used not only in organic chemistry, but also in different research areas and in particular in food science.

To the best of our knowledge, there is no review on the utilization of these techniques in

the field of food science. Therefore, the main focus of this review is firstly on the fundamental theory of NOESY (nuclear Overhauser enhancement spectroscopy) and ROESY, avoiding deep mathematical details because this is out of the scope of this review paper. We attempted to shed light on these practical methods for food scientists in order to gain a better understanding into the development of novel food products. Secondly, we discussed the latest specific applications (in food science) of these two techniques in the study of structural elucidation, conformational changes, and inclusion confirmation of functional compounds. Finally, new insights in the current and prospective applications of these techniques are provided.

2. Theory

2.1. Nuclear Overhauser Effect (NOE)

The nuclear Overhauser enhancement, also indicated as nuclear Overhauser effect, concerns the net change of signal intensity from the enhanced spin (I) due to the relaxation of a perturbed spin (S) that is dipole-dipole coupled to the first spin (Jung et al., 2000). In other words, the cross relaxation of I due to dipole-dipole interaction either between bonded nuclei or in the close vicinity of each other (< 5 Å) results in an alteration of the spin population of I (Attaur et al., 2016, Claridge, 2016b, Kumar and Rani Grace, 2017, Vögeli, 2014). Hereby, perturbation of spin S from its equilibrium population might originate from saturation (equalizing the spin population differences) or inversion of a resonance (inverting the population differences across the transitions). The result can be visualized through a 2D-spectrum called nuclear Overhauser effect spectroscopy (NOESY). There are additional cross peaks (arising from the NOE effect) besides many expected NOESY diagonal peaks (corresponding to the 1D-spectrum peaks). Large molecules tend to tumble more slowly (relax faster) in solution, which means that nuclear Overhauser effect interactions need more time to be developed. On the other hand, small molecules tumble more quickly in solution (relax slower); the movement of the nuclei allows a significant development of dipolar interactions. The result is that NOESY cross peaks may be too weak to be observed. Because the cross peaks in NOESY spectra emerge from spatial interactions, this type of spectroscopy is particularly well suited to investigate stereochemical relationships in a molecule. In fact, the aim of the NOESY NMR technique is to identify spins undergoing cross-relaxation and also to measure the cross-relaxation rates.

Typically, nuclei can have two types of magnetic interactions or couplings: dipole-dipole (D) interaction (direct) and spin-spin interaction (indirect), which is known as scalar or J-coupling. In terms of the NOE, the dipolar couplings that occur through space magnetic interactions will be concerned (Claridge, 2016b).

Theoretically, NOE is defined as the variation of a resonance when the equilibrium of spin populations is interfered with spin transitions of another. The magnitude of this transition is stated as a relative intensity change according to the following equation (Gil and Navarro-Vazquez, 2017):

Equation 1

$$N_I(S) = \frac{I - I_0}{I_0} \times 100\ (\%)$$

where $N_I(S)$ shows the NOE of spin I once it has been perturbed by spin S, I is the intensity in the presence of NOE, and I_0 is the equilibrium intensity. The enhancement is referred to the intensity variation that can be potentially either negative or positive depending on the motional characteristics (molecular size and solvent viscosity), sign of the spin's gyromagnetic ratio, and the spectrometer frequency. Typically, NOE occurs when longitudinal spin relaxation leads to the redistribution of the spin populations by transfer from one nuclei spin population to the other resulting in perturbation. This would be as a result of a stimulus i.e. the fluctuation of the magnetic field at the frequency relative to the transition generated by molecular motion (Claridge, 2016b, Marco-Rius et al., 2014). The rate of the relaxation is a function of the rotational correlation time (τ_c), an average time needed for a molecule to rotate fully through an angle of 1 rad around any axis (Spyros and Dais, 2012). Thus, molecules with a faster tumbling rate will have a short correlation time, whereas slower tumbling molecules have accordingly a longer correlation time. Claridge (2016a) suggested an approximate relation between the correlation time and molecular weight (MW, in Da) as follows:

Equation 2

$$\tau_c \approx MW \times 10^{-12}(\mathrm{s})$$

The power presented within a molecular motion leading to the transitions is called the spectral density (Rule and Hitchens, 2006). For the transition mechanisms in the relaxation processes, two possible relaxation processes (W0 and W2) can theoretically be

distinguished. The complete explanation of the energy states and spin transition is out of the scope of this article and full details can be found in a book chapter by Gil and Navarro-Vazquez (2017). The spectral density can exhibit useful information about how the relaxation rates (W0 and W2) change against tumbling rates. Typically, a relatively higher energy route (W2) belongs to the molecules with the faster tumbling rate in a solution and thereby demonstrate positive NOEs. On the other hand, slow tumbling rate molecules promote the W0 rather than W2 leading to negative NOEs (Spyros and Dais, 2012). As the relaxation rate (W) is inversely proportional to the internuclear separation distance (r^6), it may cause a rapid reduction in NOE with increasing distance. Thus, the NOE can be most likely observed when the distance between the protons is about 5 to 6 Å. Moreover, the relaxation rate is directly dependent on the gyromagnetic ratio of the two spins. So, variable rates might be obtained if the system is heteronuclear.

In the final 2-D NOE spectrum, there are two types of peaks known as diagonal and cross peaks. Diagonal peaks indicate the proton peaks observed in the 1-D spectrum, whereas the cross peaks indicate protons which are spatially close showing NOE interactions between the correlated spins (< 5 Å) (Gil and Navarro-Vazquez, 2017, Claridge, 2016b). In NOESY cross peaks, dipolar relaxation and exchange contributions cannot be distinguished as both contribute and have the same sign.

Practically, NOESY measurements can be performed in two common forms (i.e. transient or steady-state), known as two-dimensional NOESY and NOE difference techniques, respectively. The former case is typically suitable for small (MW < 1000 Da) and large molecules (MW > 2000 Da), in which the NOEs are positive and negative, respectively, while the inversion of a target generates transient NOEs. On the other hand, NOE difference can be applied for small molecules by generation of steady-state or equilibrium NOEs from saturation of a target with positive NOEs (Claridge, 2016a, Kumosinski et al., 1991).

Whereas steady-state NOE measurements are suitable for molecules that tumble rapidly in a solution, larger molecules or small molecules in a viscous solution that tumble slowly can be studied with the transient NOE technique. On the other hand, molecules with intermediate molecular weight (MW = 1000 to 2000 Da) and tumbling rates between the abovementioned conditions may have no or very weak NOE signals. Typically, NOESY is used as a homonuclear ^{1}H technique. In this method, direct dipolar couplings provide the

primary means of cross-relaxation and spins undergoing cross-relaxation are those which are close to each other in space. Thus, the cross peaks of a NOESY spectrum indicate that protons are close to each other in space. For instance, those which rely on J-coupling to provide spin-spin correlation and cross peaks, indicate that their protons are close to each other e.g. by means of molecular bonds. The basic NOESY succession consists of three $\pi/2$ pulses. The first pulse creates transverse spin magnetization. This process happens during the evolution time (t_1), which increments during the course of the 2D experiment. The second pulse produces longitudinal magnetization that is equal to the transverse magnetization component orthogonal to the pulse direction. Thus, the basic idea is to produce an initial situation for mixing time (τ_m), the time elapsed when a target resonance is monitored after initial inversion prior to the observation pulse and acquisition as a function of exchange time (Canet, 2018, Claridge, 2016b, Gil and Navarro-Vazquez, 2017). The third pulse creates transverse magnetization from the remaining longitudinal magnetization. Acquisition immediately begins after the third pulse, and the transverse magnetization is observed as a function of time (t_2). The NOESY spectrum is generated by 2D Fourier transform with respect to t_1 and t_2. Axial peaks, which originate from magnetization, have been relaxed during τ_m and can also be removed by an appropriate phase cycling. NOESY spectra can be obtained in 2D absorption mode. Occasionally, correlation spectroscopy-type artefacts appear in the NOESY spectrum. However, it is easy to identify them by their anti-phase multiple structure (Winning et al., 2007).

NOESY suffers from a detrimental effect known as spin diffusion. Spin diffusion occurs when other spins exist in close vicinity of the desired spins, which affect the magnetization process leading to the creation of negative cross peaks (Poveda and Jimenez-Barbero, 1998). Rotating frame NOE techniques may offer an appropriate solution for this problem.

2.2. Rotating-frame nuclear Overhauser effect spectroscopy (ROESY)

Rotating frame nuclear Overhauser effect spectroscopy (ROESY) is similar to NOESY, but the initial state is different. ROESY is sometimes called "cross relaxation appropriate for minimolecules emulated by locked spins" (CAMELSPIN). Instead of observing cross relaxation from an initial state of z-magnetization, the equilibrium magnetization is rotated to the x-axis and then spin-locked by an external magnetic field, so it does not precess. This method is useful for certain molecules of which their rotational correlation time is in the range where the nuclear Overhauser effect is too weak to be detected, usually molecules

with a molecular weight around 1000-2000 Da. With respect to this range, several types of molecules can be considered, including small molecule surfactants, glycosides, peptides, macro-cyclic natural compounds, dimers of diterpenes, triterpenes, steroids, and large alkaloids (Gil and Navarro-Vazquez, 2017).

In other words, ROESY and NOESY do not have a similar dependency on the correlation time and the cross-relaxation rate constant. Therefore, a different τ_m should be set during the pulse sequences (Chary and Govil, 2008). The pulse sequence of ROESY is basically similar to the pulse sequence of homonuclear 2D experiments, which is a transverse magnetization after a $\pi/2$ ^{1}H excitation followed by applying a spin lock period during τ_m resulting in the detection of the proton resonances.

The selection of the appropriate mixing time for both NOESY and ROESY is critical. With a short τ_m there would not be enough time for the NOE peaks to be observable or they are weak. By contrast, at relatively longer times, the intensity peaks would be decayed through relaxation. It should be taken into consideration that ROESY grows faster than NOESY and therefore a shorter τ_m (300 - 400 ms) may be chosen (Gil and Navarro-Vazquez, 2017). The readers are referred to Cavanagh et al. (2007) and Claridge (2016b) for the detailed explanation about the experimental parameters and the choice of an optimum mixing time.

NOE intensities go from positive to negative as the correlation time increases, becoming close to zero, whereas in ROESY the cross-relaxation rate constant is always positive (Figure 1). ROE and NOE have the same power for small molecules (around +38%). In terms of larger molecules, NOE intensity values become bigger giving maximum intensity (negative values), as compared to ROE. As can be seen in Figure 1, for medium size molecules, the ROE is still positive and maximum, while the NOE goes theoretically to zero. Although these values are not zero in practice, very small intensities will be recorded which makes the interpretation impossible.

Unlike the NOESY cross peaks, dipolar relaxation peaks appear with a negative sign in the ROE cross peaks, while the proton exchange peaks are positive, similar to the diagonal peaks (Sandström and Kenne, 2006).

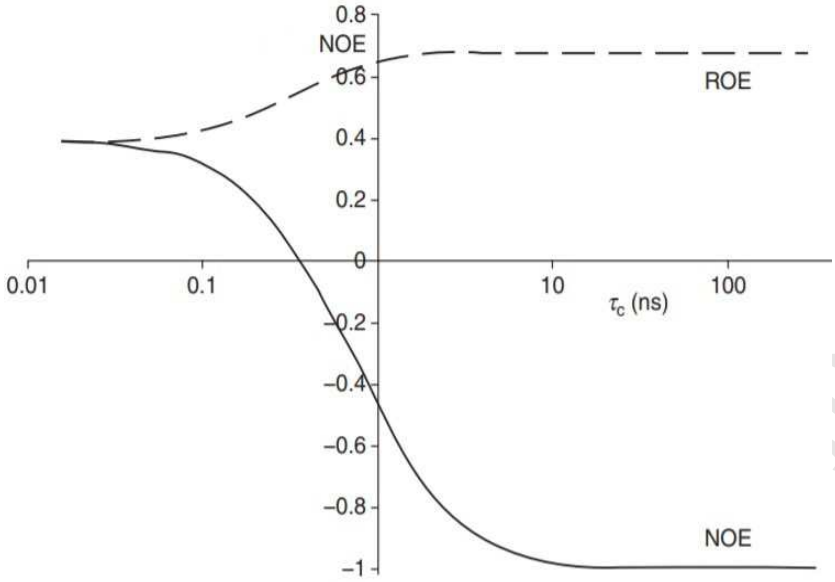


Figure 1. NOE (full line) and ROE(broken line) magnitudes as a function of molecular tumbling rate (τ_c), reprinted with permission from Williamson (2009).

Similar to other techniques, NOESY and ROESY also have some practical drawbacks that should be considered. For instance, spin diffusion in large molecular weight molecules (macromolecules) or lack of longitudinal cross relaxation can potentially interfere with the NOE results (Allard et al., 1997). Although ROE suffers less from spin diffusion (direct ROE cross peaks have opposite sign as three-spin effect cross peaks), homonuclear Hartman-Hahn transfer of magnetization might be a problem for such quantitative studies. However, off-resonance ROESY could be a solution (Allard et al., 1997).

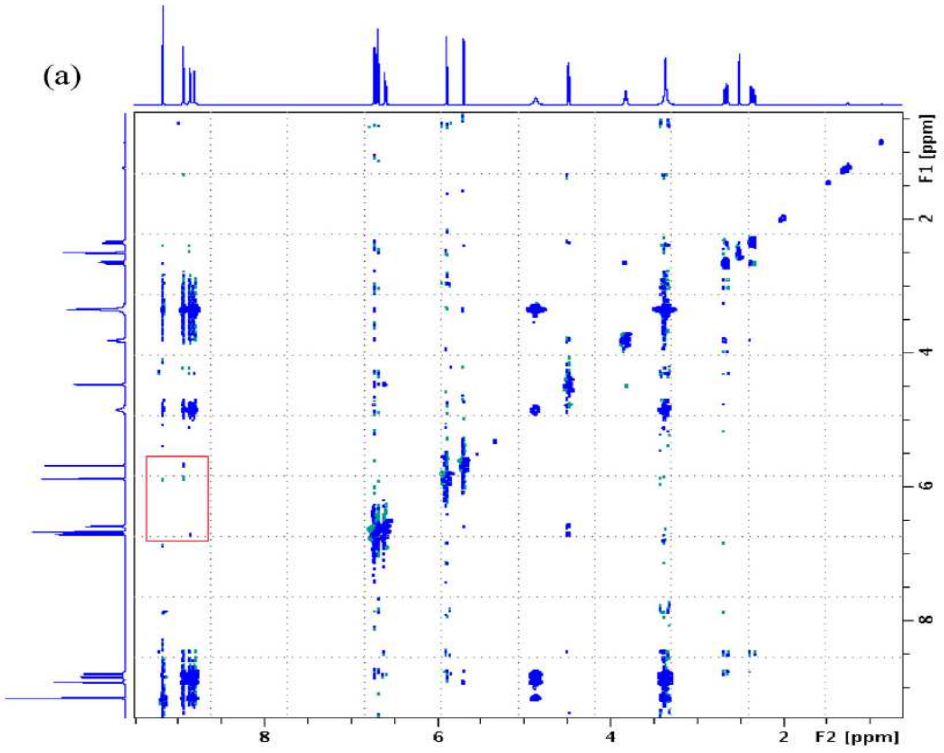
3. NOESY and ROESY Applications

Typically, these techniques can be employed to study the spatial influence and relation between nuclei of many compounds (Belloque and Ramos, 1999).

3.1. Structure elucidation

The structural characterization of organic compounds as well as of the secondary, tertiary, or quaternary structure of biological micro- and macro-molecules can be identified using 1-D or 2-D NOESY (Lau and Abdullah, 2017). For instance, NOESY is helpful to reveal the nature of glycosylic linkages between sugar residues (Cui, 2005) or intra- and inter-residue connectivity of molecules (Ai et al., 2016). Catechin polyphenol compounds are of particular interest due to their contribution to health benefits, which is attributed to the biological activity related to the hydroxyl groups in their chemical structure (Sedaghat Doost et al., 2018c). Chemical shift fingerprinting of the substituted catechins was used to

241 determine the structure profile of these compounds by Hong et al. (2017). The recorded
242 NOESY spectrum of the modified (+)-catechin hydrate is presented in Figure 2-a. The cross
243 peaks region of the protons within the hydroxyl groups is highlighted by a red square and
244 the enlarged view is shown in Figure 2-b. It can be seen that the –OH group attached to C7
245 had a correlation associated with the protons of C6 and C8. Similarly, a cross peak was
246 observed, which shows the correlation of the hydroxyl hydrogen attached to C5 and the
247 proton connected to C6. The weaker contribution was attributed to the fact that there is
248 only one proton in the close vicinity of –OH-C5, which is H-C6, as can be seen from the
249 presented chemical structure in Figure2-b (Hong et al., 2017).
250
251



252

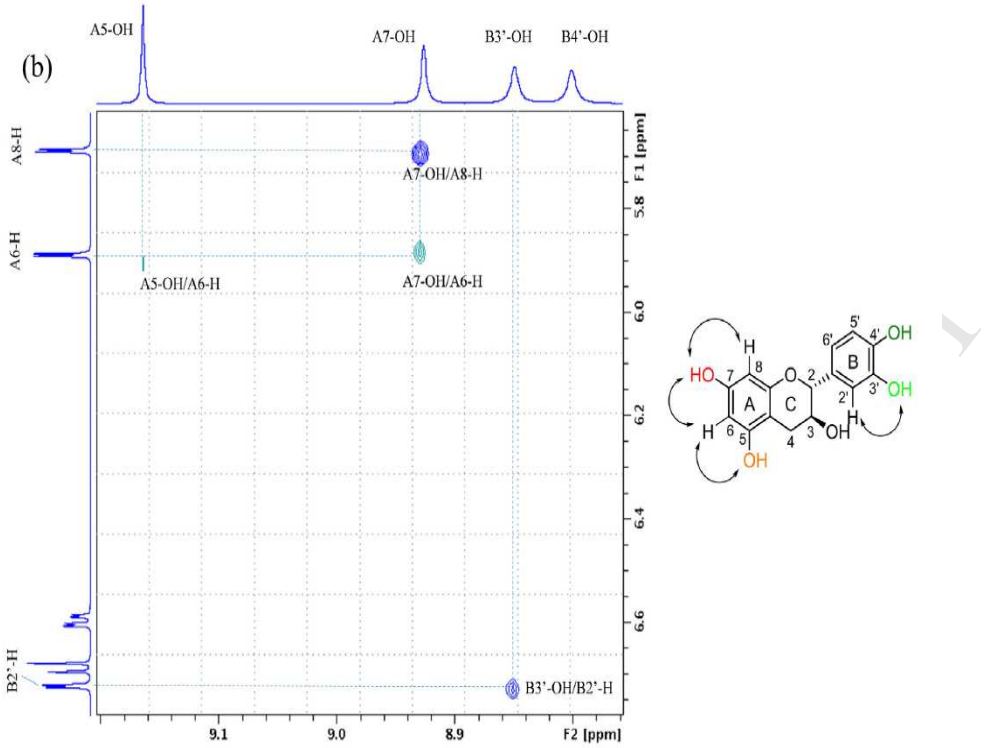


Figure 2. The full (a) and enlarged (highlighted area by red rectangle in the full spectrum, b) NOESY spectrum of catechin. The NOESY correlation of the hydroxyl protons is also shown in the chemical structure (b). Reprinted from Hong et al. (2017) with permission from Elsevier.

In a different study, the stereochemistry of goitrogenic 1,3-oxazolidine-2-thione derivatives, food compounds that may give (thyreo)toxicity to cruciferous vegetables, from Brassicales taxa was inferred by both NOESY and ROESY as complementary techniques to other NMR methods, as well as by selective homonuclear decoupling experiments (Radulović et al., 2017). Flavonoids are another group of food constituents which can chemically have an inter-flavonoid linkage that is susceptible to cleavage under different (e.g. acidic or alkaline) conditions. Different NMR techniques, including NOESY and ROESY can be employed to identify the inter-flavonoid interaction as well as the type of the upper and lower monomer units in order to recognize procyanidins from prodelphinidins (Teixeira et al., 2016, Esatbeyoglu et al., 2013).

In addition to the important role of the chemical structure of bioactive compounds for their absorption, their configuration is also believed to affect their bioavailability (Rein et al., 2013). For instance, it is shown that the S configuration of hesperitin-7-glucoside is more bioavailable than the R configuration (Lévèques et al., 2012). For a better understanding of the stereochemistry of such functional ingredients, NOESY and ROESY complementary techniques can be useful. For instance, twenty seven main bioactive compounds of *Ziziphus*

jujuba were isolated and their relative stereochemistry was established based on the NOESY correlation peaks (Bai et al., 2016). Based on the NOESY spectrum analysis, either *cis* or *trans* configurations were designated to the double bond present within the structure of some studied compounds. Secondary metabolite terpene compounds such as geranylphenol, mainly found in marine organisms, have been proved to be able to exert an antifungal effect (Taborga et al., 2017). Recently, the chemical structure of some geranylphenol compounds was evaluated by Taborga et al. (2017) who involved 1D NOESY correlations in the chemical structure evaluation.

Since protobassic acid saponins present in *Sapotaceae* family plants are reported to have cytotoxicity and antifungal properties, Chen et al. (2017) studied their isolation from the kernels of *Palaquium formosanum*, which are thought to be effective as a prostate anticancer. In this study, different analysis methods were used, including NMR techniques. Together with those NMR techniques, NOESY spectrum interpretation revealed that there was an equilibrium between two conformations of the 3' -deglucosylated 10 compound. In fact, the determination of the structure of a new compound plays a key role to predict its functional properties, which can be elaborated using NOESY or ROESY as complementary techniques. To this end, Zeraik et al. (2016) conducted a comprehensive work to elucidate the structure of a potential functional food ingredient from *Spondias tuberosa* fruit combining various methods, including 2D NOESY.

A full list of recent articles published in food science related journals that used NOESY to elucidate the chemical structure of food compounds is given in Table 1.

ROESY spectra were acquired by Masullo et al. (2017) with a 400 ms mixture time at 600 MHz to confirm the chemical structure of the extracted phenolic antioxidants from hazelnut shells. It was evident from the intensity signals that there was a correlation between the hydroxyl protons located in the same orientation, which ultimately resulted in a final structure assignment together with other NMR analyses.

The exploration of new functional food sources has been a challenge to food scientists. New lanostane-type bioactive compounds were found and characterized in the edible portion of sea cucumbers by Elbandy et al. (2014). In their study, the relative stereochemical structure of the isolated non-sulphated triterpene glycosides was elaborated using ROESY with the aid of other NMR techniques. For instance, the α – configuration of the oligosaccharide sequence and their attachment points were obtained

by interpretation of ROESY spectra. Iridoid monoterpenes, another type of functional compounds, mainly found in Cornacae family plants were isolated and identified with different NMR techniques, including ROESY (Kucharska et al., 2015). The information about the glycosilation position within the anthocyanin structure was thought to be worthwhile for color stability assessment of maqui anthocyanins (Brauch et al., 2017). ROESY revealed that the glucosyl moiety had a linkage to the delphinidin C-5 position in the studied anthocyanin.

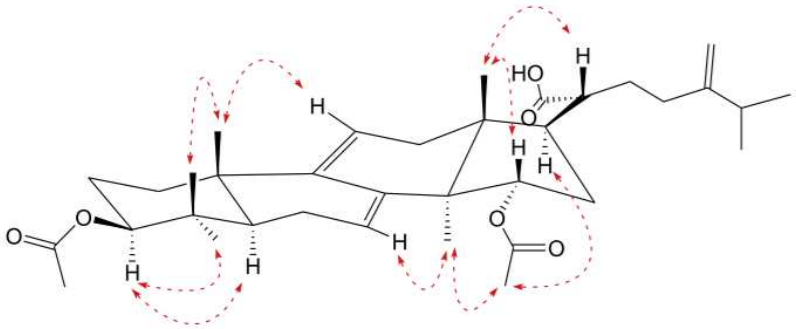
315

316 *Table 1. Relevant examples of the structure elucidation of functional food ingredients based on NOESY.*

Subject of the study	Reference
Arabinan-rich rhamnogalacturonan-I extracted from flaxseed	Ding et al. (2015)
Arabinoxylan purified and isolated from *Plantago asiatica L.* seeds	Yin et al. (2016)
Structural determination of catechin-(4-8)-dimer derivatives	Hayashi et al. (2018)
Non-starch polysaccharide extracted from roots of ginseng	Guo et al. (2015)
crude polysaccharide extracted from leaf skin of *Aloe barbadensis Miller* (*Aloe vera*)	Shi et al. (2017)
Prebiotic activity of mannanoligosaccharides isolated from palm kernel cake	Kalidas et al. (2017)
α-1, 6-linked galactomannan extracted from natural *Cordyceps sinensis*	Wang et al. (2017)
water-soluble heteropolysaccharide (TAPB1) purified from *Tremella aurantialba*	Du et al. (2015)
Isolation of a novel polysaccharide from *Boletus edulis*	Zhang et al. (2014a)
exopolysaccharides of Lactobacillus casei LC2W characterization	Ai et al. (2016)
A novel two-step enzymatic synthesis of Blastose	Miranda-Molina et al. (2017)
Neuroprotective sesquiterpenes isolated from *Petasites japonicus*	Wang et al. (2013)
α-Glucosidase inhibitor from *Buthus martensi* Karsch	Kim (2013)
Structure elucidation of *Baeckea frutescens* as an aromatic shrub	Jia et al. (2014)
New triterpene saponins from flowers of *Impatiens balsamina L.*	Li et al. (2017)
Isolation of a new caffeic acid derivative from *Tithonia diversifolia*	Pantoja Pulido et al. (2017)

3.2. Conformation and configuration analyses

NOESY and ROESY can be applied as complementary techniques to establish not only the conformational changes of small and large molecules but also configurational variations depending on the distances between nuclei, molecular motion speed, and the capability of the functional groups in rotation (Efimov et al., 2016). The spectrum obtained from NOE cross relaxation of the nuclear spins can be exploited to assign a conformation or configuration. As the conformation of a chemical compound determines its unique physical, chemical, and biological characteristics, it is substantial to distinguish the conformation of a compound, particularly an unknown compound (Carey and Sundberg, 2007). Similarly, different configurations of a chemical compound may have a significant influence on its physicochemical properties. For instance, the functional properties of a polysaccharide (such as the nutritional, technological, and physiological features) vary depending on the fact whether the polysaccharide has an α- or β-configuration (Hu et al., 2017). The NOE spectrum as complementary data to 1-D ^{1}H and ^{13}C as well as 2D HMQC (Heteronuclear Multiple-Quantum Correlation) NMR spectra was interpreted to determine the configuration of a glucan, an alkali-soluble polysaccharide extracted from an edible mushroom (Hu et al., 2017). In another study, NOESY spectra combined with x-ray diffraction data were used to establish the configuration of new cucurbitane-type glycosides extracted from the fruits of *Momordica charantia* (Zhang et al., 2014b). Similarly, the relative configuration of an ethanol extract of the sclerotia of *Poria cocos* was determined using NMR and high resolution mass spectrometry analysis (Lee et al., 2017). The relative configuration of some extracts was assigned by analysis of their NOESY spectra, the coupling constants, and ^{1}H NMR chemical shifts. For instance, as the dashed arrows display in Figure 3, a β-orientation was designated to compound 2 based on the correlation between H-15 and H-18, as well as the correlation between an acetyl proton with H-30 and H-17.



2

Figure 3. NOESY proton correlations (dashed arrows) of compound 2 in theethanol extract of the sclerotia of Poria cocos. Reprinted from Lee et al. (2017) with permission from Elsevier.

Carotenoids have different health benefits by decreasing the risk of diseases and among them, allene carotenoids showed an anti-carcinogenic activity in mammalians by triggering apoptosis in certain tumor cell lines (Krinsky and Johnson, 2005, Agócs et al., 2018). Recently, the relative configuration (axial and equatorial arrangements) of the isolated allene carotenoids from mamey using acetone with subsequent saponification was determined by the interpretation of the ROESY spectrum (Agócs et al., 2018). Previous research has shown that specific functional foods can inhibit the degeneration of human neurons leading to Alzheimer's disease. In a study performed by Xu et al. (2016), two new functional sesquiterpenoids were isolated and different conformations as well as configurations of an edible sesquiterpenoid were deduced using NOESY experiments. Owing to the precise information obtained from the NOESY technique combined with 3D modelling, a normal chair and envelope conformation was assigned to these sesquiterpenoids.

A list of recent articles related to food compounds that used NOESY to determine the structural conformation and configuration is given in Table 2.

368
369

Table 2. Overview of recent research to analyze the conformation and configuration of food components using NOESY.

Subject of the study	Reference (year)
Sesquiterpenoids from *Petasites japonicus*	Xu et al. (2016)
Jasmonoid glucosides, sesquiterpenes and coumarins from the fruit of *Clausena lansium*	Xu et al. (2014)
Anti-inflammatory effect of *birsonimadiol* from seeds of *Byrsonima Crassifolia*	Pérez Gutiérrez (2016)
Sesquiterpene lactones and scopoletins from *Artemisia scoparia Waldst. & Kit.*	Cho et al. (2016)
Isolation and characterization of a polysaccharide from the fruiting bodies of *sanghuang* mushroom (*Phellinus Baumii Pilát*)	Ge et al. (2013)

3.3. Inclusion complexes

Another practical application of these techniques is to confirm the interaction of compounds via complexation. They can be potentially employed as a direct proof for spatial proximity between two molecules.

For instance, inclusion complexes of anethole (AN), a flavouring agent in bakery, sweets, and alcoholic beverages, as a guest molecule within cyclodextrins (CD) as host molecules were studied using 2D ROESY (Kfoury et al., 2014). In this work, they proved that anethole penetrated the CD cavity via its ethylene group leading to the creation of complexes of the AN aromatic ring in two different orientations within the CD cavity. Indeed, NOE cross peaks indicated that AN molecules had an internal interaction with the CD cavity without outside binding, which confirmed that AN was complexed inside the CD cavity. Likewise, in another work, Jahed et al. (2014) showed that this technique was capable to confirm the formation of complexes between the host and guest molecules; the complex of β-cyclodextrin and curcumin was evaluated via NOE cross peaks (Figure 4) as well as by changes in the chemical shift of internal protons. The results indicated that curcumin formed a complex with β-cyclodextrin via its hydrophobic aromatic ring which promoted the solubility of curcumin. This conclusion was derived from the presence of cross peaks between the H-3 proton of β-CD and the protons from the aromatic ring of curcumin as shown in Figure 4.

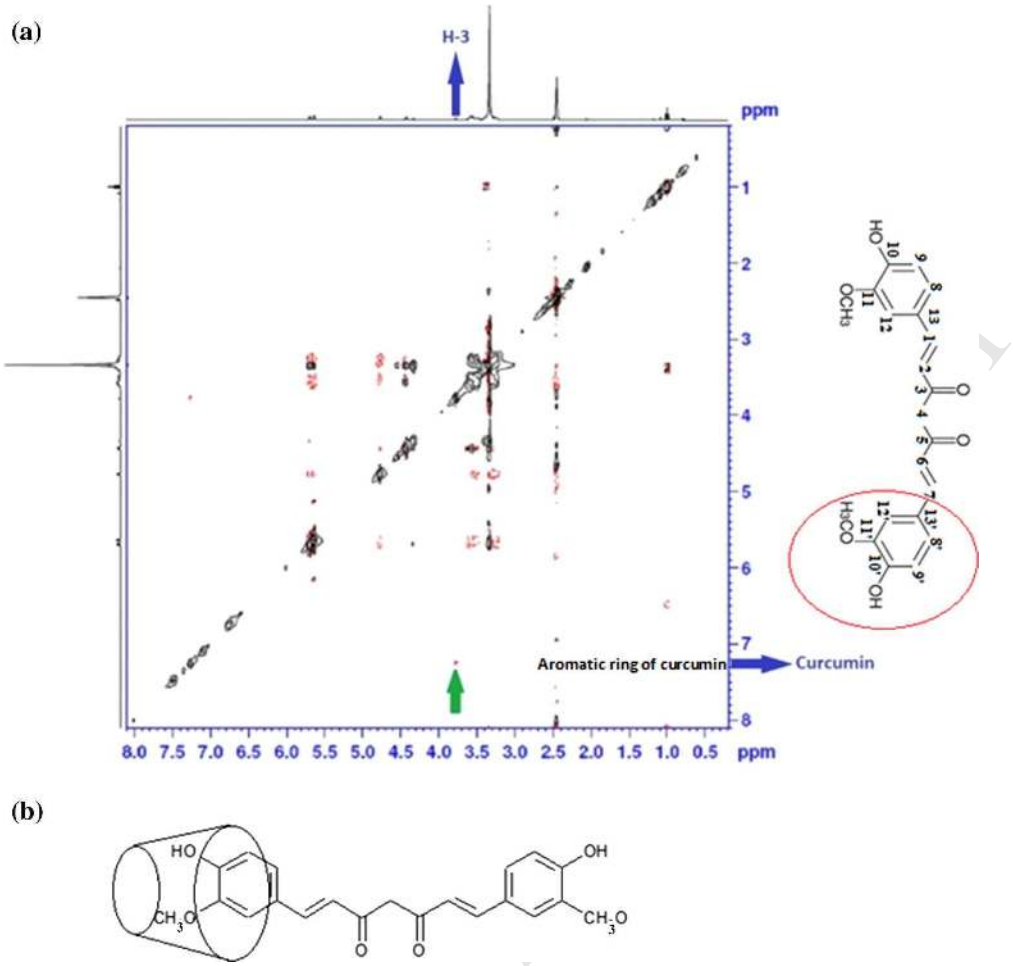


Figure 4. Inclusion complex ROESY peaks (a) and schematic chemical structure (b) of curcumin incorporated within β-cyclodextrin. Reprinted from Jahed, Zarrabi, Bordbar, & Hafezi (2014) with permission from Elsevier.

The utilization of this technique could potentially provide us new insights in the functional mechanism of host molecules. For instance, in the abovementioned work, one of the proposed aims for encapsulation of curcumin within β-cyclodextrin was to enhance its water solubility. Therefore, obtaining information about chemical and physical complexes and knowledge about which part of the molecule is specifically involved in the interaction of a guest molecule with other molecules may help to explore new delivery systems.

Recently, there has been a great demand for consumption of food products containing natural ingredients, including essential oils (EOs). The latter are hydrophobic volatile compounds that are increasingly attracting interest due to their antimicrobial and antioxidant properties (Sedaghat Doost et al., 2018b, Sedaghat Doost et al., 2019).

Eugenol EO, primarily extracted from the flower buds of clove or cinnamon, was used in a study by Gong et al. (2016) as a guest molecule inside the β-cyclodextrin host site. These eugenol - β-CD complexes were intended to be used as an antifungal to preserve litchi fruits during postharvest storage and transportation. In order to confirm the formation of

complexes and the interaction sites of the molecules, 2D NOESY spectra were acquired, which revealed that eugenol interacted via the methyl group of its aromatic ring with the lipophilic site inside the cavity. Similarly, carvacrol as a major constituent of some EOs (Sedaghat Doost et al., 2018a, Sedaghat Doost et al., 2017) was encapsulated into β-CD not only to promote its solubility but also to prolong the release and thereby enhancing its functional features, such as its antimicrobial activity (Lavoine et al., 2014). This inclusion was confirmed through 2D-NOE peaks. Lavoine et al. (2014) reported the dipolar interaction between the aromatic group protons of carvacrol and the H-5 proton of β-CD in its cavity.

CD, a biodegradable oligosaccharide, has been the subject of several studies owing to its special chemical structure which enables it to make an inclusion complex with different organic compounds. This increases their water solubility and therefore bioavailability (Birck et al., 2016). Depth penetration of sodium benzoate, a preservative, inside the hydroxypropyl-β-cyclodextrin cavity within a polyvinyl alcohol-citric acid film was established using 2D ROESY. Birck et al. (2016) concluded from the proton correlations obtained by ROESY cross peaks that sodium benzoate was successfully placed inside the cavity and the Job's method analysis showed that the stoichiometry of the interaction between hydroxypropyl-β-cyclodextrin and sodium benzoate was 1:1.

Another study carried out by Matencio et al. (2017) showed the host-guest inclusion complex of oxyresveratrol, a stilbenoid extracted from mulberry fruits, with a modified CD using NOESY. Similar to the previously mentioned studies, the analysis of the correlated protons exhibited that the oxyresveratrol aromatic ring was involved in an interaction with the internal cavity.

In another case, the interaction of caffeine with an aqueous solution of di-O-caffeoylquinic acid was studied. In this research, analyses of NOESY and ROESY peaks revealed that the three methyl groups of caffeine interacted with the aromatic groups of di-O-caffeoylquinic acid isomers (D'Amelio et al., 2015).

3.4. Other applications

An alternative application is using NOESY as a tool for solvent suppression, primarily in metabonomic studies. Most biological or medicinal samples have to be analyzed in water, which makes the observation of relevant peaks challenging. Although in most of the cases

deuterated water (5-10 %) is added to do a lock spin, the prominent proton peak of water prevents the efficient detection of solutes with low concentration (i.e. mM range) (Claridge, 2016c, Kew et al., 2017). In order to suppress the proton peak of water (i.e. reduce the magnitude of the solvent resonance) before it can be detected by the receiver, several approaches have been suggested, including saturation, zero net excitation, and destruction using pulsed field gradients of the water resonance (Cavanagh et al., 2007, Ross et al., 2007).

The non-selective 1-D NOESY-based approach, which is obtained at short mixing times (usually 0 to 10 ms), is commonly utilized in metabonomics for solvent suppression. Recently, digested food samples were studied to figure out the amino acid fraction that is solubilized at different digestion levels (Ménard et al., 2018). The final amino acid composition of the digested proteins through the infant gastrointestinal tract was analyzed using different techniques, to determine the extent of digestion. Solvent suppression was applied using first increment NOESY NMR and the relative proton intensity of the solubilized products of proteolysis was obtained.

The composition of the complex mixture of gut microbiota could be studied to establish low molecular weight metabolites in fecal waters and study the relation between the host metabolism and constituents of gut microbiota (Le Roy et al., 2015). To analyze these types of samples using NMR, water suppression had to be applied. A presaturation approach using NOESY was carried out prior to recording metabolic and fecal water spectra (Matysik et al., 2016). It has been shown that the microbial activity during fermentation of dairy products can be investigated using mass spectrometry or NMR techniques. The metabolites of set-yoghurt containing different proteolytic strains of *Streptococcus thermophilus* co-cultured with *Lactobacillus delbrueckii* subsp. *Bulgaricus* was studied using ^{1}H NMR spectroscopy, but 1-D NOESY was utilized for solvent suppression (Settachaimongkon et al., 2014). Dellarosa et al. (2016) also used a NOESY sequence to suppress the signal of residual water in order to study the final metabolic profiling of fresh apples treated with pulsed electric fields using high resolution ^{1}H NMR spectroscopy.

4. Conclusions and future research

The basic principles of two NMR techniques including nuclear Overhauser and rotating-frame Overhauser effect and their applications in food science were thoroughly reviewed.

Whereas NOESY is mostly appropriate for relatively large and small molecules, ROESY could be a more efficient choice for mid-sized molecules (1000-3000 Da). The chemical structure of novel extracts or compounds could be elucidated using these methods. Moreover, the conformation, configuration, as well as inclusion complex formation of chemical structures can be determined using these NMR techniques in combination with other analysis tools. Practically, only very low amounts of the sample (microliter) are needed, which provides a big advantage because of the cost or the limited availability of specific samples. However, NMR techniques are generally not considered as highly sensitive methods, and hence require a relatively high concentration (preferably in the mM range). Moreover, in the selection of the method and the practical parameters, some detrimental effects such as spin diffusion should be taken into consideration.

In the future, these techniques can be potentially significant analysis tools for the development of novel food products. The valuable information about the chemical structure of food constituents obtained by these methods could substantially proceed the production of innovative foods. For instance, protein and polysaccharide interaction has been widely studied as it has a number of different applications, e.g. in food science. Despite the complex chemical structure of proteins like whey protein isolate, which is a mixture of different proteins, useful information of the interaction sites with polysaccharides might be obtained through these techniques rather than present computer simulations. The conformation and binding site of surfactants on the surface of oil droplets as well as the effect of environmental conditions on their chemical structure may be another future perspective that can be considered using NOESY and ROESY NMR methods in food science.

Acknowledgements

References

AGÓCS, A., MURILLO, E., TURCSI, E., BÉNI, S., DARCSI, A., SZAPPANOS, Á., KURTÁN, T. & DELI, J. 2018. Isolation of allene carotenoids from mamey. *Journal of Food Composition and Analysis,* 65**,** 1-5.

AI, L., GUO, Q., DING, H., GUO, B., CHEN, W. & CUI, S. W. 2016. Structure characterization of exopolysaccharides from Lactobacillus casei LC2W from skim milk. *Food Hydrocolloids,* 56**,** 134-143.

ALLARD, P., HELGSTRAND, M. & HÄRD, T. 1997. A method for simulation of NOESY, ROESY, and Off-Resonance ROESY spectra. *Journal of Magnetic Resonance,* 129**,** 19-29.

ARNOLD, J. T., DHARMATTI, S. S. & PACKARD, M. E. 1951. Chemical effects on nuclear induction signals from organic compounds. *Journal of Chemical Physics,* 19**,** 507-507.

ATTAUR, R., CHOUDHARY, M. I. & ATIATUL, W. 2016. Chapter 6 - Nuclear Overhauser Effect. *Solving Problems with NMR Spectroscopy (Second Edition).* Boston: Academic Press.

BAI, L., ZHANG, H., LIU, Q., ZHAO, Y., CUI, X., GUO, S., ZHANG, L., HO, C.-T. & BAI, N. 2016. Chemical characterization of the main bioactive constituents from fruits of Ziziphus jujuba. *Food & Function,* 7**,** 2870-2877.

BELLOQUE, J. & RAMOS, M. 1999. Application of NMR spectroscopy to milk and dairy products. *Trends in Food Science & Technology,* 10**,** 313-320.

BIRCK, C., DEGOUTIN, S., MATON, M., NEUT, C., BRIA, M., MOREAU, M., FRICOTEAUX, F., MIRI, V. & BACQUET, M. 2016. Antimicrobial citric acid/poly(vinyl alcohol) crosslinked films: Effect of cyclodextrin and sodium benzoate on the antimicrobial activity. *LWT - Food Science and Technology,* 68**,** 27-35.

BOTHNER-BY, A. A., STEPHENS, R. L., LEE, J., WARREN, C. D. & JEANLOZ, R. W. 1984. Structure determination of a tetrasaccharide: transient nuclear Overhauser effects in the rotating frame. *Journal of the American Chemical Society,* 106**,** 811-813.

BRAUCH, J. E., REUTER, L., CONRAD, J., VOGEL, H., SCHWEIGGERT, R. M. & CARLE, R. 2017. Characterization of anthocyanins in novel Chilean maqui berry clones by HPLC–DAD–ESI/MSn and NMR-spectroscopy. *Journal of Food Composition and Analysis,* 58**,** 16-22.

CANET, D. 2018. Chapter 1 Introduction to Nuclear Spin Cross-relaxation and Cross-correlation Phenomena in Liquids. *Cross-relaxation and Cross-correlation Parameters in NMR: Molecular Approaches.* The Royal Society of Chemistry.

CAREY, F. A. & SUNDBERG, R. J. 2007. Stereochemistry, Conformation, and Stereoselectivity. *In:* CAREY, F. A. & SUNDBERG, R. J. (eds.) *Advanced Organic Chemistry: Part A: Structure and Mechanisms.* Boston, MA: Springer US.

CARVER, T. R. & SLICHTER, C. P. 1953. Polarization of nuclear spins in metals. *Physical Review,* 92**,** 212-213.

CAVANAGH, J., FAIRBROTHER, W. J., PALMER, A. G., RANCE, M. & SKELTON, N. J. 2007. CHAPTER 6 - EXPERIMENTAL 1H NMR METHODS. *In:* CAVANAGH, J., FAIRBROTHER, W. J., PALMER, A. G., RANCE, M. & SKELTON, N. J. (eds.) *Protein NMR Spectroscopy (Second Edition).* Burlington: Academic Press.

CHARY, K. V. R. & GOVIL, G. 2008. *NMR in Biological Systems,* Netherlands, Springer Netherlands.

CHEN, H.-Y., LIU, J.-F., TSAI, S.-F., LIN, Y.-L. & LEE, S.-S. 2017. Cytotoxic protobassic acid saponins from the kernels of Palaquium formosanum. *Journal of Food and Drug Analysis,* 26**,** 557-564.

CHO, J.-Y., JEONG, S.-J., LEE, H. L., PARK, K.-H., HWANG, D. Y., PARK, S.-Y., LEE, Y. G., MOON, J.-H. & HAM, K.-S. 2016. Sesquiterpene lactones and scopoletins from Artemisia scoparia Waldst. & Kit. and their angiotensin I-converting enzyme inhibitory activities. *Food Science and Biotechnology,* 25**,** 1701-1708.

CLARIDGE, T. D. W. 2016a. Chapter 6 - Correlations Through the Chemical Bond I: Homonuclear Shift Correlation. *High-Resolution NMR Techniques in Organic Chemistry (Third Edition).* Boston: Elsevier.

CLARIDGE, T. D. W. 2016b. Chapter 9 - Correlations Through Space: The Nuclear Overhauser Effect. *High-Resolution NMR Techniques in Organic Chemistry (Third Edition).* Boston: Elsevier.

CLARIDGE, T. D. W. 2016c. Chapter 12 - Experimental Methods. *High-Resolution NMR Techniques in Organic Chemistry (Third Edition).* Boston: Elsevier.

CUI, S. W. 2005. *Determination of Linkage Pattern: Methylation Analysis, Reductive Cleavage, and Peroxidation* CRC Press.

D'AMELIO, N., PAPAMOKOS, G., DREYER, J., CARLONI, P. & NAVARINI, L. 2015. NMR studies of hetero-association of caffeine with di-O-caffeoylquinic acid isomers in aqueous solution. *Food Biophysics,* 10**,** 235-243.

DELLAROSA, N., TAPPI, S., RAGNI, L., LAGHI, L., ROCCULI, P. & DALLA ROSA, M. 2016. Metabolic response of fresh-cut apples induced by pulsed electric fields. *Innovative Food Science & Emerging Technologies,* 38**,** 356-364.

DING, H. H., CUI, S. W., GOFF, H. D., CHEN, J., WANG, Q. & HAN, N. F. 2015. Arabinan-rich rhamnogalacturonan-I from flaxseed kernel cell wall. *Food Hydrocolloids,* 47**,** 158-167.

DU, X., ZHANG, Y., MU, H., LV, Z., YANG, Y. & ZHANG, J. 2015. Structural elucidation and antioxidant activity of a novel polysaccharide (TAPB1) from Tremella aurantialba. *Food Hydrocolloids,* 43**,** 459-464.

EFIMOV, S. V., KHODOV, I. A., RATKOVA, E. L., KISELEV, M. G., BERGER, S. & KLOCHKOV, V. V. 2016. Detailed NOESY/T-ROESY analysis as an effective method for eliminating spin diffusion from 2D NOE spectra of small flexible molecules. *Journal of Molecular Structure,* 1104**,** 63-69.

ELBANDY, M., RHO, J. R. & AFIFI, R. 2014. Analysis of saponins as bioactive zoochemicals from the marine functional food sea cucumber Bohadschia cousteaui. *European Food Research and Technology,* 238**,** 937-955.

ESATBEYOGLU, T., WRAY, V. & WINTERHALTER, P. 2013. Identification of two novel prodelphinidin A-type dimers from roasted hazelnut skins (Corylus avellana L.). *Journal of Agricultural and Food Chemistry,* 61**,** 12640-12645.

GE, Q., MAO, J.-W., ZHANG, A.-Q., WANG, Y.-J. & SUN, P.-L. 2013. Purification, chemical characterization, and antioxidant activity of a polysaccharide from the fruiting bodies of sanghuang mushroom (Phellinus baumii Pilát). *Food Science and Biotechnology,* 22**,** 301-307.

GIL, R. R. & NAVARRO-VAZQUEZ, A. 2017. Chapter 1 Application of the Nuclear Overhauser Effect to the Structural Elucidation of Natural Products. *Modern NMR Approaches to the Structure Elucidation of Natural Products: Volume 2: Data Acquisition and Applications to Compound Classes.* The Royal Society of Chemistry.

GONG, L., LI, T., CHEN, F., DUAN, X., YUAN, Y., ZHANG, D. & JIANG, Y. 2016. An inclusion complex of eugenol into β-cyclodextrin: Preparation, and physicochemical and antifungal characterization. *Food Chemistry,* 196**,** 324-330.

GUO, Q., CUI, S. W., KANG, J., DING, H., WANG, Q. & WANG, C. 2015. Non-starch polysaccharides from American ginseng: physicochemical investigation and structural characterization. *Food Hydrocolloids,* 44**,** 320-327.

HAYASHI, S., NAKANO, K. & YANASE, E. 2018. Investigation of color-deepening phenomenon in catechin-(4→8)-dimer as a proanthocyanidin model and structural determination of its derivatives by oxidation. *Food Chemistry,* 239**,** 1126-1133.

HONG, S., WU, S., CAI, H., WANG, Y. & LIU, S. 2017. Instant structure profiling of substituted catechins by chemical shift fingerprint of hydrogens of phenolic hydroxyl groups. *Journal of Functional Foods,* 37**,** 58-65.

HU, T., HUANG, Q., WONG, K., YANG, H., GAN, J. & LI, Y. 2017. A hyperbranched β-d-glucan with compact coil conformation from Lignosus rhinocerotis sclerotia. *Food Chemistry,* 225**,** 267-275.

JAHED, V., ZARRABI, A., BORDBAR, A.-K. & HAFEZI, M. S. 2014. NMR (1H, ROESY) spectroscopic and molecular modelling investigations of supramolecular complex of β-cyclodextrin and

curcumin. *Food Chemistry,* 165**,** 241-246.
JIA, B.-X., ZENG, X.-L., REN, F.-X., JIA, L., CHEN, X.-Q., YANG, J., LIU, H.-M. & WANG, Q. 2014. Baeckeins F–I, four novel C-methylated biflavonoids from the roots of Baeckea frutescens and their anti-inflammatory activities. *Food Chemistry,* 155**,** 31-37.
JUNG, D.-M., DE ROPP, J. S. & EBELER, S. E. 2000. Study of interactions between food phenolics and aromatic flavors using one- and two-dimensional 1H NMR spectroscopy. *Journal of Agricultural and Food Chemistry,* 48**,** 407-412.
KALIDAS, N. R., SAMINATHAN, M., ISMAIL, I. S., ABAS, F., MAITY, P., ISLAM, S. S., MANSHOOR, N. & SHAARI, K. 2017. Structural characterization and evaluation of prebiotic activity of oil palm kernel cake mannanoligosaccharides. *Food Chemistry,* 234**,** 348-355.
KEW, W., BELL, N. G. A., GOODALL, I. & UHRÍN, D. 2017. Advanced solvent signal suppression for the acquisition of 1D and 2D NMR spectra of Scotch Whisky. *Magnetic Resonance in Chemistry,* 55**,** 785-796.
KFOURY, M., AUEZOVA, L., GREIGE-GERGES, H., RUELLAN, S. & FOURMENTIN, S. 2014. Cyclodextrin, an efficient tool for trans-anethole encapsulation: Chromatographic, spectroscopic, thermal and structural studies. *Food Chemistry,* 164**,** 454-461.
KIM, S.-D. 2013. α-Glucosidase inhibitor from Buthus martensi Karsch. *Food Chemistry,* 136**,** 297-300.
KRINSKY, N. I. & JOHNSON, E. J. 2005. Carotenoid actions and their relation to health and disease. *Molecular Aspects of Medicine,* 26**,** 459-516.
KUCHARSKA, A. Z., SZUMNY, A., SOKÓŁ-ŁĘTOWSKA, A., PIÓRECKI, N. & KLYMENKO, S. V. 2015. Iridoids and anthocyanins in cornelian cherry (Cornus mas L.) cultivars. *Journal of Food Composition and Analysis,* 40**,** 95-102.
KUMAR, A. & RANI GRACE, R. C. 2017. Nuclear Overhauser Effect A2 - Lindon, John C. *In:* TRANTER, G. E. & KOPPENAAL, D. W. (eds.) *Encyclopedia of Spectroscopy and Spectrometry (Third Edition).* Oxford: Academic Press.
KUMOSINSKI, T. F., BROWN, E. M. & FARRELL, H. M. 1991. Molecular modeling in food research: technology and techniques. *Trends in Food Science & Technology,* 2**,** 110-115.
LAU, B. F. & ABDULLAH, N. 2017. Bioprospecting of Lentinus squarrosulus Mont., an underutilized wild edible mushroom, as a potential source of functional ingredients: A review. *Trends in Food Science & Technology,* 61**,** 116-131.
LAVOINE, N., GIVORD, C., TABARY, N., DESLOGES, I., MARTEL, B. & BRAS, J. 2014. Elaboration of a new antibacterial bio-nano-material for food-packaging by synergistic action of cyclodextrin and microfibrillated cellulose. *Innovative Food Science & Emerging Technologies,* 26**,** 330-340.
LE ROY, C. I., ŠTŠEPETOVA, J., SEPP, E., SONGISEPP, E., CLAUS, S. P. & MIKELSAAR, M. 2015. New insights into the impact of Lactobacillus population on host-bacteria metabolic interplay. *Oncotarget,* 6**,** 30545-30556.
LEE, S., LEE, D., LEE, S. O., RYU, J.-Y., CHOI, S.-Z., KANG, K. S. & KIM, K. H. 2017. Anti-inflammatory activity of the sclerotia of edible fungus, Poria cocos Wolf and their active lanostane triterpenoids. *Journal of Functional Foods,* 32**,** 27-36.
LÉVÈQUES, A., ACTIS-GORETTA, L., REIN, M. J., WILLIAMSON, G., DIONISI, F. & GIUFFRIDA, F. 2012. UPLC–MS/MS quantification of total hesperetin and hesperetin enantiomers in biological matrices. *Journal of Pharmaceutical and Biomedical Analysis,* 57**,** 1-6.
LI, Q., CAO, J., YUAN, W., LI, M., YANG, L., SUN, Y., WANG, X. & ZHAO, Y. 2017. New triterpene saponins from flowers of Impatiens balsamina L. and their anti-hepatic fibrosis activity. *Journal of Functional Foods,* 33**,** 188-193.
MARCO-RIUS, I., BOHNDIEK, S. E., KETTUNEN, M. I., LARKIN, T. J., BASHARAT, M., SEELEY, C. & BRINDLE, K. M. 2014. Quantitation of a spin polarization-induced nuclear Overhauser effect (SPINOE) between a hyperpolarized 13C-labeled cell metabolite and water protons. *Contrast Media & Molecular Imaging,* 9**,** 182-186.

MASULLO, M., CERULLI, A., MARI, A., DE SOUZA SANTOS, C. C., PIZZA, C. & PIACENTE, S. 2017. LC-MS profiling highlights hazelnut (Nocciola di Giffoni PGI) shells as a byproduct rich in antioxidant phenolics. *Food Research International,* 101**,** 180-187.

MATENCIO, A., GARCÍA-CARMONA, F. & LÓPEZ-NICOLÁS, J. M. 2017. The inclusion complex of oxyresveratrol in modified cyclodextrins: A thermodynamic, structural, physicochemical, fluorescent and computational study. *Food Chemistry,* 232**,** 177-184.

MATYSIK, S., LE ROY, C. I., LIEBISCH, G. & CLAUS, S. P. 2016. Metabolomics of fecal samples: A practical consideration. *Trends in Food Science & Technology,* 57**,** 244-255.

MÉNARD, O., BOURLIEU, C., DE OLIVEIRA, S. C., DELLAROSA, N., LAGHI, L., CARRIÈRE, F., CAPOZZI, F., DUPONT, D. & DEGLAIRE, A. 2018. A first step towards a consensus static in vitro model for simulating full-term infant digestion. *Food Chemistry,* 240**,** 338-345.

MIRANDA-MOLINA, A., CASTILLO, E. & LOPEZ MUNGUIA, A. 2017. A novel two-step enzymatic synthesis of blastose, a β-d-fructofuranosyl-(2↔6)-d-glucopyranose sucrose analogue. *Food Chemistry,* 227**,** 202-210.

PANTOJA PULIDO, K. D., COLMENARES DULCEY, A. J. & ISAZA MARTÍNEZ, J. H. 2017. New caffeic acid derivative from Tithonia diversifolia (Hemsl.) A. Gray butanolic extract and its antioxidant activity. *Food and Chemical Toxicology,* 109**,** 1079-1085.

PÉREZ GUTIÉRREZ, R. M. 2016. Anti-inflammatory effect of birsonimadiol from seeds of Byrsonima crassifolia. *Food Science and Biotechnology,* 25**,** 561-566.

POVEDA, A. & JIMENEZ-BARBERO, J. 1998. NMR studies of carbohydrate-protein interactions in solution. *Chemical Society Reviews,* 27**,** 133-144.

RADULOVIĆ, N. S., TODOROVSKA, M. M., ZLATKOVIĆ, D. B., STOJANOVIĆ, N. M. & RANDJELOVIĆ, P. J. 2017. Two goitrogenic 1,3-oxazolidine-2-thione derivatives from Brassicales taxa: Challenging identification, occurrence and immunomodulatory effects. *Food and Chemical Toxicology,* 110**,** 94-108.

REIN, M. J., RENOUF, M., CRUZ-HERNANDEZ, C., ACTIS-GORETTA, L., THAKKAR, S. K. & DA SILVA PINTO, M. 2013. Bioavailability of bioactive food compounds: a challenging journey to bioefficacy. *British journal of clinical pharmacology,* 75**,** 588-602.

ROSS, A., SCHLOTTERBECK, G., DIETERLE, F. & SENN, H. 2007. Chapter 3 - NMR Spectroscopy Techniques for Application to Metabonomics. *In:* LINDON, J. C., NICHOLSON, J. K. & HOLMES, E. (eds.) *The Handbook of Metabonomics and Metabolomics.* Amsterdam: Elsevier Science B.V.

RULE, G. S. & HITCHENS, T. K. 2006. *Fundamentals of Protein NMR Spectroscopy*, Springer Netherlands.

SANDSTRÖM, C. & KENNE, L. 2006. Hydroxy Protons in Structural Studies of Carbohydrates by NMR Spectroscopy. *NMR Spectroscopy and Computer Modeling of Carbohydrates.* American Chemical Society.

SEDAGHAT DOOST, A., DEVLIEGHERE, F., DIRCKX, A. & VAN DER MEEREN, P. 2018a. Fabrication of Origanum compactum essential oil nanoemulsions stabilized using Quillaja Saponin biosurfactant. *Journal of Food Processing and Preservation,* 42**,** e13668.

SEDAGHAT DOOST, A., DEWETTINCK, K., DEVLIEGHERE, F. & VAN DER MEEREN, P. 2018b. Influence of non-ionic emulsifier type on the stability of cinnamaldehyde nanoemulsions: A comparison of polysorbate 80 and hydrophobically modified inulin. *Food Chemistry,* 258**,** 237-244.

SEDAGHAT DOOST, A., MUHAMMAD, D. R. A., STEVENS, C. V., DEWETTINCK, K. & VAN DER MEEREN, P. 2018c. Fabrication and characterization of quercetin loaded almond gum-shellac nanoparticles prepared by antisolvent precipitation. *Food Hydrocolloids,* 83**,** 190-201.

SEDAGHAT DOOST, A., NIKBAKHT NASRABADI, M., KASSOZI, V., DEWETTINCK, K., STEVENS, C. V. & VAN DER MEEREN, P. 2019. Pickering stabilization of thymol through green emulsification using soluble fraction of almond gum – Whey protein isolate nano-complexes. *Food*

Hydrocolloids, 88**,** 218-227.
SEDAGHAT DOOST, A., SINNAEVE, D., DE NEVE, L. & VAN DER MEEREN, P. 2017. Influence of non-ionic surfactant type on the salt sensitivity of oregano oil-in-water emulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* 525**,** 38-48.
SETTACHAIMONGKON, S., NOUT, M. J. R., ANTUNES FERNANDES, E. C., HETTINGA, K. A., VERVOORT, J. M., VAN HOOIJDONK, T. C. M., ZWIETERING, M. H., SMID, E. J. & VAN VALENBERG, H. J. F. 2014. Influence of different proteolytic strains of Streptococcus thermophilus in co-culture with Lactobacillus delbrueckii subsp. bulgaricus on the metabolite profile of set-yoghurt. *International Journal of Food Microbiology,* 177**,** 29-36.
SHI, X.-D., NIE, S.-P., YIN, J.-Y., QUE, Z.-Q., ZHANG, L.-J. & HUANG, X.-J. 2017. Polysaccharide from leaf skin of Aloe barbadensis Miller: Part I. Extraction, fractionation, physicochemical properties and structural characterization. *Food Hydrocolloids,* 73**,** 176-183.
SPYROS, A. & DAIS, P. 2012. NMR Spectroscopy in Food Analysis. *Royal Society of Chemistry***,** P001-329.
TABORGA, L., SORTINO, M., CARRASCO, H., BUTASSI, E., ZACCHINO, S. & ESPINOZA, L. 2017. Antifungal toxicity of linear geranylphenol. Influence of oxigenate substituents. *Food and Chemical Toxicology,* 109**,** 827-835.
TEIXEIRA, N., MATEUS, N. & DE FREITAS, V. 2016. Updating the research on prodelphinidins from dietary sources. *Food Research International,* 85**,** 170-181.
VÖGELI, B. 2014. The nuclear Overhauser effect from a quantitative perspective. *Progress in Nuclear Magnetic Resonance Spectroscopy,* 78**,** 1-46.
WANG, J., NIE, S., CHEN, S., PHILLIPS, A. O., PHILLIPS, G. O., LI, Y., XIE, M. & CUI, S. W. 2017. Structural characterization of an α-1, 6-linked galactomannan from natural Cordyceps sinensis. *Food Hydrocolloids,* 78**,** 77-91.
WANG, S., JIN, D.-Q., XIE, C., WANG, H., WANG, M., XU, J. & GUO, Y. 2013. Isolation, characterization, and neuroprotective activities of sesquiterpenes from Petasites japonicus. *Food Chemistry,* 141**,** 2075-2082.
WILLIAMSON, M. P. 2009. Chapter 3 Applications of the NOE in Molecular Biology. *Annual Reports on NMR Spectroscopy.* Academic Press.
WINNING, H., VIERECK, N., NØRGAARD, L., LARSEN, J. & ENGELSEN, S. B. 2007. Quantification of the degree of blockiness in pectins using 1H NMR spectroscopy and chemometrics. *Food Hydrocolloids,* 21**,** 256-266.
XU, J., JI, F., CAO, X., MA, J., OHIZUMI, Y., LEE, D. & GUO, Y. 2016. Sesquiterpenoids from an edible plant Petasites japonicus and their promoting effects on neurite outgrowth. *Journal of Functional Foods,* 22**,** 291-299.
XU, X., XIE, H. & WEI, X. 2014. Jasmonoid glucosides, sesquiterpenes and coumarins from the fruit of Clausena lansium. *LWT - Food Science and Technology,* 59**,** 65-69.
YIN, J.-Y., WANG, J.-Q., LIN, H.-X., XIE, M.-Y. & NIE, S.-P. 2016. Fractionation, physicochemical properties and structural features of non-arabinoxylan polysaccharide from the seeds of Plantago asiatica L. *Food Hydrocolloids,* 55**,** 128-135.
ZERAIK, M. L., QUEIROZ, E. F., MARCOURT, L., CICLET, O., CASTRO-GAMBOA, I., SILVA, D. H. S., CUENDET, M., DA SILVA BOLZANI, V. & WOLFENDER, J.-L. 2016. Antioxidants, quinone reductase inducers and acetylcholinesterase inhibitors from Spondias tuberosa fruits. *Journal of Functional Foods,* 21**,** 396-405.
ZHANG, A.-Q., LIU, Y., XIAO, N.-N., ZHANG, Y. & SUN, P.-L. 2014a. Structural investigation of a novel heteropolysaccharide from the fruiting bodies of Boletus edulis. *Food Chemistry,* 146**,** 334-338.
ZHANG, L.-J., LIAW, C.-C., HSIAO, P.-C., HUANG, H.-C., LIN, M.-J., LIN, Z.-H., HSU, F.-L. & KUO, Y.-H. 2014b. Cucurbitane-type glycosides from the fruits of Momordica charantia and their hypoglycaemic and cytotoxic activities. *Journal of Functional Foods,* 6**,** 564-574.

Highlights

- Principle and applications of nuclear and rotating-frame Overhauser effects.
- Characterization of stereochemical structure using the NMR techniques.
- The complex inclusion of novel compounds could be investigated.

.