Metabolomics (2019) 15:44
https://doi.org/10.1007/s11306-019-1504-7

REVIEW ARTICLE

The magic angle view to food: magic-angle spinning (MAS) NMR spectroscopy in food science

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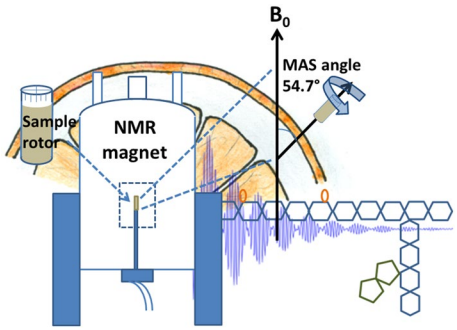
Received: 21 December 2018 / Accepted: 4 March 2019 / Published online: 13 March 2019

Abstract

Nuclear Magnetic Resonance (NMR) spectroscopy has been used in food science and nutritional studies for decades and is one of the major analytical platforms in metabolomics. Many foods are solid or at least semi-solid, which denotes that the molecular motions are restricted as opposed to in pure liquids. While the majority of NMR spectroscopy is performed on liquid samples and a solid material gives rise to constraints in terms of many chemical analyses, the magic angle thrillingly enables the application of NMR spectroscopy also on semi-solid and solid materials. This paper attempts to review how magic-angle spinning (MAS) NMR is used from 'farm-to-fork' in food science.

Graphical abstract



Keywords Semi-solid food characterization · Food composition · Foodomics · Food metabolites · Meat · Dairy · Plant-based food · Taste compounds · Cheese · Food authenticity · Intact tissue · Food structure · Fruit ripening · Grain filling, vegetables, plant biochemistry

Abbreviations and definitions

2D	Two-dimensional. Term used for techniques, which give data plotted in a space defined by two frequency axes rather than one and with the intensities constituting a third dimension
CMP	Comprehensive multiphase NMR combining both ^{1}H HR-MAS, diffusion restriction and ^{13}C CP MAS type of experiments in one probe

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11306-019-1504-7) contains supplementary material, which is available to authorized users.

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CP	Cross polarization. CP is technique employed in solid-state NMR to transfer magnetization from one type of spin to another type of spin through space. This is achieved by applying a pulse simultaneously on the two different spins
CPMG	Carr–Purcell–Meiboom–Gill. CPMG is a pulse sequence that utilizes a T_2 relaxation filter to remove or attenuate broad resonances from of molecules with a short T_2 relaxation. This enables broadening of macromolecules beyond detection, allowing improved detection of small molecules
DANTE	Delays alternating with nutations for tailored excitation (Morris and Freeman 1978).
DNP	Dynamic nuclear polarisation. Highly sensitive NMR technique where spectra are recorded under hyperpolarisation of the sample
DSC	Differential scanning calorimetry
FTIR	Fourier transform InfraRed spectroscopy
GABA	γ-Aminobutyric acid
GC	Gas chromatography
HCA	Hierarchical cluster analysis. HCA is method that is used to classify and cluster samples or variables based on their similarities and dissimilarities
HR	High resolution
HR-MAS	High resolution MAS. A term defined by the vendor for NMR probe heads optimized for ^{1}H NMRof gel-state samples (not crystalline, but soft solids, swollen materials or gels), where standard liquid pulse sequences (1D and 2D, HSQC, TOCSY) can be applied under magic angle spinning conditions
HPLC	High pressure liquid chromatography
HSQC	Heteronuclear single quantum correlation. HSQC is a 2D experiment used in NMR spectroscopy. In a ^{1}H-^{13}C HSQC experiment, information about the correlation between the aliphatic carbon and its attached protons are obtained, which can facilitate in spectral assignment
iMQC	Hadamard encoded intermolecular multiple-quantum coherence
MAS	Magic angle spinning. MAS is a technique used in solid-state NMR spectroscopy and consists of spinning the sample at the magic angle θ_m (54.74°) with respect to the direction of the magnetic field to enhance the spectral resolution
MVDA	Multivariate data analysis. MVDA is typically used to explore variations and trends in data across a high number of variables. As NMR spectral data consist of a high number of variables, MVDA is a useful tool to explore NMR spectral data
NOESY	Nuclear Overhauser enhancement spectroscopy. An NMR experiment that takes advantage of the Nuclear Overhauser effect, consisting of dipole–dipole interactions through space
O-PLS	Orthogonal partial least squares. O-PLS is an adaption of PLS that separates the systematic variation in X into variation that is related or unrelated to Y
PASS	Phase-altered spinning sideband. Scheme for two-dimensional sideband separation in MAS NMR. The scheme can produce spinning sideband-free solid-state NMR spectra (Antzutkin et al. 1995)
PCA	Principal component analysis
PHORMAT	Phase-corrected magic angle turning. Scheme for two-dimensional sideband separation in MAS NMR. The scheme can produce spinning sideband-free solid-state NMR spectra (Hu et al. 1995)
PLS	Partial least squares projections to latent structures is the supervised extension of PCA where the data matrix, X, is related to Y by regression
PLS-DA	Partial least squares discriminant analysis. PLS-DA is a supervised method that uses a categorical response variable
PMMA	Poly(methyl methacrylate). Polymer material used for MAS rotors
POM	Polyoxymethylene. Polymer material used for MAS rotors

PRISE	Proton relaxation induced spectral-editing. A ^{13}C cross-polarization experiment that takes advantage of differences in the ^{1}H relaxation to obtain information on molecular dynamics. If multiple components are present in the proton relaxation processes, proton magnetisation can be prepared in such a way that the magnetisation of protons having different relaxation times is separated (Tang et al. 2000)
qNMR	Quantitative NMR. qNMR refers to the use of NMR to determine the exact concentration of one or more chemical species
RINEPT	: Refocused-insensitive nuclei enhanced by polarization transfer. RINEPT is technique employed in solution NMR to elucidates scalar (or J-) couplings between 1H and 13C nuclei. The technique has occasionally been adopted to solid-state NMR to transfer magnetization from one type of spin to another type of spin through bonds (Arnold et al. 2015)
Shimming	The procedure for optimizing the magnetic field homogeneity prior to acquisition of a spectrum
SP	Single pulse. The most basic NMR experiment is called a single pulse experiment. The experiment begins with the system at equilibrium and thus magnetization is oriented along the *z* axis. The first step is a 90° x pulse, which means that an excitation field is applied along the *x* axis by one of the radio frequency coils
SWET	Secure water suppression enhanced through T1 effects. Modified version of the WET pulse sequence where each selective pulse is broken up in the DANTE fashion, inserting bipolar pulsed-field gradients in the delays (Wu and Otting 2005)
TOCSY	Total correlation spectroscopy. TOCSY is a 2D experiment that enables the detection of cross peaks of coupled protons. Cross peaks are observed both for nuclei which are directly coupled and also between nuclei which are connected by a chain of couplings. This makes it useful for identifying the larger interconnected networks of spin couplings.
WATERGATE	Water suppression by gradient-tailored excitation. In WATERGATE the gradient echo sequence combines a selective 180-degrees radiofrequency pulse and two field gradient pulses to achieve a highly selective and effective water suppression (Piotto et al. 1992).
WET	Water suppression enhanced through T1 effects. This pulse sequence uses shaped, selective pulses and pulsed magnetic field gradients to suppress one or more solvent signals. The WET scheme is based on a series of water-selective excitation pulses followed by pulsed field gradients to defocus the transverse water magnetization (Smallcombe et al. 1995).
WISE	Wide line separation NMR. WISE is an NMR experiment based on the use of cross-polarization to characterize molecular dynamics (Schmidt-Rohr et al. 1992).
XRD	X-ray diffraction

1 Introduction

The magic angle is an exact defined angle of 54.7356° that was discovered by Andrew et al. (1958). Andrew et al. (1958) described that at the magic angle of 54.7° the nuclear dipole–dipole interactions, chemical shift anisotropy and variations in magnetic susceptibility, which cause line broadening in the NMR spectrum, are eradicated. Consequently, the NMR features of a solid material that is spun in an angle of 54.7° with respect to the direction of magnetic field resembles the NMR features of a liquid sample. In liquids, most of these interactions causing line broadening will average out because of the rapid time-averaged molecular motions that occur, and magic-angle spinning (MAS) is mimicking this condition existing in liquids. Typically, magnets used for MAS NMR experiments have field strengths between 7.0 T (300 MHz ^{1}H resonance frequency) and 14.0 T (600 MHz ^{1}H resonance frequency). Often spinning rates in the KHz range are applied in the MAS NMR experiment. Spinning rates typically depend on the specific application and type of experiment. Spinning rates of up to 10 KHz can easily be achieved in modern probes for HR MAS, which is typically used for metabolite profiling of semi-solid samples. In solid-state MAS experiments, which is typically applied for molecular mobility studies of real solids (e.g. crystalline powders), even higher spinning rates may be employed. The spinning of the sample is achieved via an air turbine mechanism that uses either air or nitrogen gas

as an air bearing. The samples are packed into a so-called rotor that typically has a diameter between 3 and 5 mm and is then sealed with a single or double end cap. The rotors are made from a number of different materials such as ceramics e.g. zirconia or polymers such as poly(methyl methacrylate) (PMMA) or polyoxymethylene (POM). The type of sample material and temperatures applied during the experiment are decisive for the type of rotor used.

Packing of rotors is somewhat cumbersome and often limits the number of samples that can be analyzed by MAS NMR. Depending on training level, it is estimated that 10–30 samples can be prepared in rotors per day. Often disposable inserts are used and the correct packing of the rotor has to ensure no void volumes/air-bubbles, which can complicate shimming and therefore result in poor spectral quality. Examples of thorough protocols for MAS NMR sample preparation are provided by Gaëlle et al. (2015) and Beckonert et al. (2010), who investigated intact marine algae and liver tissue samples, respectively. Video tutorials are also available to assist newcomers (Heath and Claus 2011, as well as Bruker BioSpin on Youtube: https://www.youtube.com/watch?v=bNFJj2g0UjI).

1.1 Types of MAS techniques

Table 1 provides an overview of the most common MAS NMR techniques. In general, MAS NMR spectroscopy can be divided into two main categories; (1) solid-state NMR spectroscopy and (2) HR-MAS NMR spectroscopy. Solid-state NMR spectroscopy includes single pulse (SP) and cross-polarization (CP) experiments and is mostly applied to "real solids", i.e. crystalline material or powders to study molecular mobilities, while HR-MAS NMR spectroscopy is mostly applied to semi-solid samples for metabolite profiling. In foods, the most common nuclei in MAS NMR include proton (^{1}H), carbon (^{13}C) and in more rare or specialized applications also phosphorous (^{31}P) and nitrogen (^{15}N).

1.1.1 ^{1}H MAS NMR

The high natural abundance of ^{1}H (99.985%) together with its high sensitivity described by its gyromagnetic ratio (γ), makes ^{1}H MAS NMR a very sensitive technique (Friebolin 1998). The most used form of ^{1}H MAS NMR in food science is the so-called ^{1}H HR-MAS NMR technique, which is applied to biological matrices by the use of special probeheads. Standard high-resolution liquid-state pulse sequences (1D as well as 2D) are typically applied on soft and semi-solid samples (not crystalline) in ^{1}H MAS NMR spectroscopy. The only obstacle attached to ^{1}H HR-MAS NMR spectroscopy is that many foods have a relatively high water content, and therefore water protons tend to dominate the spectra with resultant loss of information from ^{1}H present in less-abundant molecules. However, this dynamic range issue is easily overcome by the inclusion of water suppression techniques in the pulse sequence applied in the NMR experiment, a variety of which exist. The most common water suppression techniques applied in foods are based on presaturation or selective saturation, which applies a long and low power pulse at the frequency of the water resonance usually positioned around 4.6 ppm (Hoult 1976). In water suppression techniques such as WET and SWET suppression is achieved through T1 relaxation effects (Smallcombe et al. 1995; Wu and Otting 2005) and WATERGATE employs pulsed-field gradients to attenuate the water resonance (Piotto et al. 1992).

In conventional ^{1}H HR-MAS NMR experiments, spinning rates in the KHz range are typically employed. Even though MAS enables measurements on intact tissues, a major concern is the very high spinning speeds in the KHz-range which can likely promote disruption and disintegration of the sample structure and thereby also accelerate degradation processes. Thereby, the ^{1}H HR-MAS NMR analyses may not be providing a realistic picture of the sample under investigation, and methodologies to overcome this problem have therefore been developed. Wind and co-workers initiated much of this work and adapted techniques from

Table 1 Overview of the most common MAS NMR techniques

MAS technique	NMR characteristics	Fields of applications
MAS or SP MAS	^{13}C, ^{31}P, ^{23}Na – strong proton decoupling, long acquisition times, typically the best spectra are obtained on powdered samples	Crystalline samples or mixtures of amorphous and crystalline. All carbon atoms observed
CP MAS	^{13}C, ^{31}P Cross Polarisation, shorter acquisition time, typically the best spectra are obtained on powdered samples	Crystalline samples or mixtures of amorphous and crystalline. The most crystalline parts will have the sharpest line-shapes. By changing the contact time for CP dynamics of rigid parts of mixtures can be investigated
HR-MAS	^{1}H, ^{13}C, ^{31}P, HR-MAS probe head with inverse geometry, liquid-state NMR pulse sequences, 2D experiments, short acquisition time	Excellent technique for global profiling of small metabolites in soft solid samples, swollen materials or gels. Intact tissue can be analysed

traditional solid-state NMR, namely phase-altered spinning sidebands (PASS) and phase-corrected magic angle turning (PHORMAT) techniques to enable measurements on biological materials under application of considerably lower spinning speeds (Wind et al. 2001; Hu et al. 2002). With PASS MAS spinning speeds down to 30 Hz has been demonstrated on biological materials (Wind et al. 2001; Hu et al. 2002). Bertram et al. (2004d) reported a study on rabbit muscles where post mortem metabolism was followed dynamically by employing PASS ^{1}H MAS NMR at a spinning speed of 40 Hz.

Recently, attempts to acquire MAS NMR spectra of biological materials under slow MAS conditions that relies on techniques adapted from liquid-state NMR have also evolved. By a technique that involves optimization of the sample preparation as well as a modern relaxation filter adapted from liquid-state NMR, Andre et al. (2014) demonstrated a methodology that enabled the acquisition of high-resolution ^{1}H HR-MAS NMR spectra of biological material (fish eggs) at a spinning speed of 400 Hz. The most common relaxation filter applied in ^{1}H HR-MAS to eliminate broad signals from larger molecules is based on the Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence (Carr and Purcell 1954; Meiboom and Gill 1958), but Andre et al. (2014) adapted an alternative relaxation filter, as CPMG-editing was found to be unsuitable for the slow spinning speeds employed. As an alternative to employing a CPMG-filter in ^{1}H HR-MAS experiments, the so-called PROJECT sequence has been developed for applications in NMR metabolomics studies (Aguilar et al. 2012; LeGuennec et al. 2017). The PROJECT sequence avoids artefacts from J-evolution.

1.1.2 Multivariate data analysis (MVDA)

^{1}H HR-MAS NMR spectroscopy of foods typically generates high-resolution spectra with a vast of metabolite resonances and multivariate data analytical approaches are pertinent to explore variations and trends in ^{1}H HR-MAS NMR data. The working horse in MVDA is principal component analysis (PCA) (Pearson 1901; Hotelling 1933). MVDA is often divided into un-supervised and supervised methods. PCA is an unsupervised projection method where the dimension of variables is reduced to a fewer number of principal components (PCs) that capture the major variation in the data. The PCs are linear combinations of the original variables and describe the variation in the data in a descending order of magnitude. PCA is a classical method and used to extract and display variations in the data matrix. PCA provides an overview of the data, revealing trends, patterns, groups and possible outliers. Hierarchical cluster analysis (HCA) is another unsupervised method that is used to classify and cluster samples or variables based on their similarities and dissimilarities (Ward 1963). HCA seeks to build a hierarchy of clusters and a cluster tree (dendrogram) is formed where the distance between clusters indicates the di(similarity).

Supervised methods are characterized by the inclusion of dependent variables, Y, containing quantitative values. Partial least squares (PLS) projections to latent structures is the supervised extension of PCA where the data matrix, X, is related to Y by a regression method to find linear relationships between the data matrix X and Y (Wold et al. 2001). PLS can be elaborated in various directions. Discriminant analysis (PLS-DA) uses a categorical response variable and is commonly used to identify differences between sample groups or classes. PLS extended to a multilevel version enables to take paired data into account and is superior for the analysis of data from a cross-over designed study (Westerhuis et al. 2010). Orthogonal PLS (O-PLS) is an adaption of PLS that separates the systematic variation in X into variation that is related or unrelated to Y (Trygg and Wold 2002). Model validation is fundamental in supervised methods, ruling out chance and substantiating the significance of the model. To learn further, several on-line resources can be recommended including http://www.models.life.ku.dk/, which includes examples and animations.

1.1.3 ^{13}C MAS NMR

Solid-state NMR spectroscopy is commonly based on the ^{13}C nucleus. The natural abundance of ^{13}C is relatively low (1.1%) (Friebolin 1998), its gyromagnetic ratio (γ) is also relatively low, however, as a result of ^{13}C being present in a vast amount of compounds and metabolites, solid-state ^{13}C MAS NMR spectroscopy has found many applications in food science. The technique commonly used is single pulse (SP) MAS and reports quantitatively on all ^{13}C present in the food, but the sensitivity is low due the low natural abundance and gyromagnetic ratio, and often hours or even days of recording is needed. Typically, the sensitivity in ^{13}C MAS NMR measurements is enhanced through so-called cross-polarization (CP) where magnetization is transferred from ^{1}H to ^{13}C. The simplest CP experiment involves initially a 90° pulse applied to the ^{1}H nuclei (99.99% natural abundance), which flips the magnetization to the XY plane. The ^{1}H and ^{13}C magnetizations are then spin-locked during a subsequent contact time pulse followed by a ^{1}H decoupling using high power decoupling during the acquisition of the ^{13}C signal. The signal enhancement that is achieved in the CP pulse experiment depends on the polarization transfer/contact time and the rotating-frame T1 relaxation of both ^{1}H and ^{13}C. The rotating-frame T1 relaxation is dependent on distance between ^{13}C and ^{1}H nuclei and the molecular motions. Therefore, the enhancement achieved will be different for C, CH, CH_2 and CH_3 groups. Consequently, CP experiments are inherently not quantitative, but can provide

useful information about molecular motions and thus be deemed a mobility resolved NMR technique (Foster et al. 1996). In general ^{13}C CP experiments will favour the most rigid/crystalline parts of the food systems. By changing the contact time from short to long, differences in the mobility of food constituents can be monitored. ^{13}C CP experiments represent the most used technique in solid-state NMR spectroscopy. More detailed descriptions of CP experiments can be found elsewhere (Conte et al. 2004). An excellent on-line resource for illustrations of the basic concepts of CP MAS NMR can be found at the Glenn Facey's blog: http://u-of-o-nmr-facility.blogspot.com/.

2 MAS NMR in vegetables and fruits

In the early 1990s the first studies on the use of MAS NMR spectroscopy to characterize intact fruits were reported. Ni and Eads (1993a) showed that ^{1}H MAS NMR analyses of grape, banana and apple enabled the detection of several sugars, acids, and lipid species, even when these occurred in separate phases. The use of MAS NMR techniques in plant-based food science can be divided in two major areas. The first area deals with the understanding of the structures and dynamics of large biopolymers of the separating layers of fruits, leaves and seeds. The second major area is the use of MAS NMR to profile metabolites of intact plant tissue, typically applied in order to follow ripening and flavour development as well as distribution of primary and secondary metabolites. Metabolite profiling is also used to document geographical authenticity as well as genotype differences. A list of studies in these areas is provided in Table 2.

2.1 Understanding the structure of outer protective layers

The protective tissue of plants serves to prevent water, osmotic and metabolite losses and to protect against physical as well as pathogen damage and hence forms insoluble barriers, which are layers of interacting polymers that are difficult to analyse in their intact form. Illustration of fruit protective layers can be found in Fig. S1.

Often the backbone units of large biomolecules in fruits, vegetables and seeds can be well characterized upon hydrolytic or enzymatic degradation by using conventional analytical techniques such as GC, HPLC, and/or liquid-state NMR spectroscopy. This is the case of the outer layer of waxes, the middle layer of cutin-wax polyesters and the rigid cell wall cellulose/pectin/lignin of e.g. lemon, tomato and potato. Deshmukh et al. (2003) investigated the branching and crosslinking of tomato cutin polyester structural units using MAS NMR. Stark and coworkers (Stark et al. 2000; Yu et al. 2006; Serra et al. 2012; Chatterjee et al. 2016) used swelling in solvents and 1D, 2D MAS NMR and non-swelling solid state using both CP and SP MAS NMR to study biomolecules and structure of tomato and potato tissue. MAS NMR data were compared with conventional liquid-state NMR data to assign chemical structures, and contact time variations and 2D experiments NOESY were used to reveal space interactions. Yu et al. (2006) achieved to reveal covalent binding from COSY and HMQC experiments.. Intriguingly, the flexibility and infection resistance between different tomato genotypes could be related to the hydrophilic/hydrophobic ratio (integrals of $(CHO + CH_2O)/(CH_2)_n$) measured by ^{13}C SP MAS NMR of cutin polyesters (Chatterjee et al. 2016), and thereby MAS NMR provided important information on relations between chemical features of the plant tissue and its resistance to infection.

Fresh citrus (*Diamante*) and lemon (*Primofiore*) fruit tissues have been examined by Mucci et al. (2013) where each part of the fruit peel: flavedo (outer layer), oil glands, the white albedo, the inner pulp and seeds were analyzed separately by HR-MAS NMR. The flavedo spectra were dominated by waxes and carbohydrates. Of the 56 different nutrients/metabolites identified, the terpenes (like: beta-pinene) were selectively found in the flavedo and oil glands, while the aldehydes like citronellal were dominant in the oil glands. No flavonoids, such as hesperidin, were observed using HR-MAS NMR, most likely due to broad unassignable peaks near the limit of detection. On the other hand, another potential bioactive alkaloid trigonelline was observed in citrus pulp. Surprisingly, when applying NMR diffusion filters to observe the larger molecules (polysaccharides/fatty acids from cutins/waxes) in the flavedo with restricted motion, the usually fast-tumbling terpenes could also be observed. This finding indicates that the terpenes are found both in the macroscopic oil glands with a high degree of mobility but also exist in a more motion-restricted form among the polymers of the protective layer. This is an example of the ability of HR MAS NMR to investigate molecules in different tissue compartments, which is challenging with any other analytical technique.

2.2 Plant biochemistry: ripening process, cultivar differences and geographical origin

The first study reporting the use of HR-MAS to study fruit ripening was conducted on banana (Ni and Eads 1993b). Ripening of mango has also been followed by ^{1}H HR-MAS (Gil et al. 2000). Later, metabolic profiling by the use of HR MAS has been applied by Pérez and coworkers to study tomatoes for tissue differentiation, fruit ripening (Pérez et al. 2010) and as markers for protection of origin (Pérez et al. 2011). Spectra of seeds were dominated by linoleic (47%) and oleic acid (28%) (Pérez et al. 2010). The use of MVDA showed metabolite trajectories during the ripening process,

Table 2 Overview of MAS NMR spectroscopic studies

Reference	Product	Objective of the study	MAS experiment employed
Plant-based products			
Choze et al. (2013)	Common bean	Discrimination of transgenic and conventional genotype	^{1}H HR-MAS NMR spectroscopy (1D)
Corsaro et al. (2015)	Mediterranean food	Geographical protection	^{1}H HR-MAS NMR spectroscopy (1D)
Delgano-Goñi et al. (2013)	Melon varieties	Quantification of glucose, fructose and sucrose in intact plant tissue	^{1}H HR-MAS NMR spectroscopy (qNMR)
Deshmukh et al. (2003)	Tomato	Demonstrate cutin polymer structure and crosslinking	^{1}H HR-MAS (1D and 2D) and CP MAS NMR spectroscopy
Gil et al. (2000)	Mango pulp	Quantification of sugars during ripening	^{1}H HR-MAS NMR spectroscopy
Mucci et al. (2013)	Citrus varieties	Identification of metabolites in each type of plant tissue	^{1}H HR-MAS NMR spectroscopy (1D and 2D)
Ni and Eads (1993a)	Grape, banana and apple	Quantification of sugars, acids and lipids	^{1}H HR-MAS NMR spectroscopy
Ni and Eads (1993b)	Ripening of banana	Quantification of sugars, acids and lipids	^{1}H HR-MAS NMR spectroscopy
Otero and Prestamo (2009)	Strawberries	Effects of pressurization	^{1}H HR-MAS NMR spectroscopy
Pérez et al. (2010)	Tomato	Method development for the study of tissue metabolite composition and ripening	^{1}H HR-MAS NMR spectroscopy (1D)
Pérez et al. (2011)	Tomato varieties	Flavor profiling of hybrids vs. non-hybrid varieties	^{1}H HR-MAS NMR spectroscopy (1D and 2D)
Ritota et al. (2010)	Sweet pepper	Discrimination of different varieties	^{1}H HR-MAS NMR spectroscopy
Ritota et al. (2012)	Garlic *(Allium sativum L.)*	Discrimination of different varieties and geographical origin	^{1}H HR-MAS NMR spectroscopy (1D and 2D)
Serra et al. (2012)	Tomato and potato (wild-type vs. mutant)	Architecture of polymers in plant protective tissue	^{1}H HR-MAS and CP MAS NMR spectroscopy
Song et al. (2016)	Rice grain cultivars	Differences between main cultivar types and influence of environmental conditions	^{1}H HR-MAS NMR Spectroscopy
Stark et al. (2000)	Tomato and potato	Structure and dynamics in fruit cuticle polyesters	^{2}H -^{13}C MAS NMR + WISE spectroscopy
Vermathen et al. (2011)	Apple	Differentiation of cultivars	^{1}H HR-MAS NMR spectroscopy
Vermathen et al. (2017)	Apple	Effects of production system (organic vs. integrated and low-input systems)	^{1}H HR-MAS NMR spectroscopy
Yu et al. (2006)	Potato	Understand intercellular adhesion-strengthened parenchyma tissues	^{1}H HR-MAS (1D and 2D) and CP MAS NMR spectroscopy
Starch-containing products			
Bardet et al. (2006)	Lettuce (*Lactusa sativa*) Garden Pea (*Pisum sativum*)	Composition and fatty acid profile of seeds	^{13}C CP MAS NMR spectroscopy
Brescia and Sacco (2006)	Durum Wheat Flour -> Dough	To follow the bread making process	^{1}H HR-MAS NMR and^{13}C CP MAS NMR spectroscopy
Calucci and Geppi (2006)	Wheat proteins, wheat durum and soft wheat flours	To follow the hydration of wheat proteins	Static ^{1}H MAS, ^{1}H HR-MAS, ^{13}C CP MAS, and ^{13}C SP MAS NMR spectroscopy
Gidley and Bociek (1985)	Rice, maize waxy maize, amylo-maize and potato starch	First reporting of MAS NMR of different types compared with X-ray powder diffraction	^{13}C CP MAS NMR spectroscopy

Table 2 (continued)

Reference	Product	Objective of the study	MAS experiment employed
Gidley (1989)	Waxy maize, amylose, wheat starch	Amylose aggregation and gelation	^{13}C CP MAS NMR spectroscopy
Larsen et al. (2013)	Native and modified Potato starch	The study of hydration effects and enzymatic modifications	^{13}C CP MAS NMR, ^{31}P SP, and CP MAS NMR spectroscopy
Mihhalevski et al. (2012)	Rye bread (sourdough raising)	Crystallisation Amylose/amylopectin	^{13}C CP MAS NMR spectroscopy
Morgan et al. (1995)	Waxy maize, amylose, wheat starch	The study of starch branching and lipid inclusion complexes in non-waxy starch	^{13}C CP and ^{13}C SP MAS NMR spectroscopy
Paris et al. (1999)	Waxy maize, amylose, wheat starch	Crystalline starch heterogeneity	^{13}C CP and ^{13}C SP MAS NMR spectroscopy
Paris et al. (2001a)	Waxy maize (amylo-pectin), linear amylose (from peas), branched starch (potato starch)	Amorphous starch heterogeneity	^{13}C CP MAS NMR spectroscopy
Paris et al. (2001b)	Amylose, amylopectin	Amorphous starch magnetisation transfer and starch heterogeneity	2D solid-state WISE NMR spectroscopy
Sacco et al. (1998)	Wheat flour	Effects of geographical origin	^{1}H HR-MAS NMR spectroscopy
Seefeldt et al. (2008)	Barley	Grain filling of barley	^{1}H HR-MAS NMR spectroscopy
Tan et al. (2007)	Native starch from rice, wheat, maize and waxy maize	Spectral decomposition for quantification of amorphous and crystalline forms	^{13}C CP MAS NMR spectroscopy
Tang and Hills (2003)	Native starch from pea, potato and corn	Hydration dynamics	^{13}C PRISE, CP MAS spectroscopy SP NMR spectroscopy
Tang and Wang (2006)	Starch granules	Review starch NMR techniques	A variety of NMR experiments
Meat products			
Bertram et al. (2003)	Porcine muscles	Study post mortem muscle glycogen degradation	^{13}C CP MAS NMR spectroscopy
Bertram et al. (2004a)	Rabbit muscles	Study post mortem energy metabolism	^{1}H and ^{13}C solid-state MAS NMR
Bertram et al. (2004b)	Porcine muscles	Mobility resolved NMR study of cell membrane constituents	^{13}C CP MAS NMR and low-field NMR ^{1}H relaxometry
Brescia et al. (2002)	Beef meat	Methodology demonstration	^{1}H HR-MAS NMR spectroscopy
Garcia-Garcia et al. (2018)	Dry-fermented sausages	To elucidate metabolite generation during ripening	^{1}H HR-MAS NMR spectroscopy
Longobardi et al. 2012	Goat meat	Metabolite characterization	^{1}H HR-MAS NMR spectroscopy
Ritota et al. (2012)	Beef meat	Differentiation of breed and muscle type	^{1}H HR-MAS NMR spectroscopy
Sacco et al. (2005)	Lamb meat	Geographical origin classification	^{1}H HR-MAS NMR spectroscopy
Shintu et al. (2007)	Dried beef meat	Geographical origin classification	^{1}H HR-MAS NMR spectroscopy
Fish products			
Aursand et al. (2008)	Salmon (*Salmo solar*)	Determine relative contents of n-3 FA, EPA and DHA	^{1}H HR-MAS NMR spectroscopy
Bankefors et al. (2011)	Salmon (*Salmo solar*)	Determine relative contents of n-3 FA, EPA and DHA	^{1}H HR-MAS NMR spectroscopy
Castejon et al. (2010)	Salmon (*Salmo solar*)	Elucidate storage-induced changes	^{1}H HR-MAS NMR spectroscopy

Table 2 (continued)

Reference	Product	Objective of the study	MAS experiment employed
Heude et al. (2015)	Brown trout, red mullet (*Mullus barbatus*), Wild sea bass (*Dicentrarchus labrax*), and wild sea bass (*Sparus aurata*)	Determine fish freshness	^{1}H HR-MAS NMR spectroscopy
Nestor et al. (2010)	Arctic char	Determine relative contents of n-3 FA, EPA and DHA	^{1}H HR-MAS NMR spectroscopy
Villa et al. (2013)	Salmon (*Salmo solar*)	Elucidate effects of irradiation	^{1}H HR-MAS NMR spectroscopy
Cheese and dairy products			
Gobet et al. (2010)	Soft cheese	Intrinsic phosphate distribution	^{31}P MAS and CP ^{31}P MAS NMR spectroscopy
Haque et al. (2015)	Milk whey protein concentrates	Solubility after storage	^{13}C CP MAS NMR spectroscopy
Lamanna et al. (2008)	Soft cheese	Packaging	^{1}H HR-MAS NMR spectroscopy (standard 1D)
Lamichhane et al. (2015)	Soft cheese	Processing parameters: rennet, starter culture and NaCl content Correlation to sensory analyses	^{1}H HR-MAS NMR spectroscopy (standard 1D and CPMG-edited)
Mazzei and Piccolo (2012)	Mozarella cheese	Geographical origin	^{1}H HR-MAS NMR spectroscopy (standard 1D, diffusion-edited and CPMG-edited)
Shintu et al. (2004)	Parmigiano Reggiano	Analytical method development	^{1}H HR-MAS NMR spectroscopy (standard 1D)
Shintu and Caldarelli (2005)	Parmigiano Reggiano	Ripening-induced changes	^{1}H HR-MAS NMR spectroscopy (standard 1D)
Shintu and Caldarelli (2006)	Emmentaler cheese	Geographical origin	^{1}H HR-MAS NMR spectroscopy (standard 1D)

and as an example, an increase in glutamate was identified going from green to red tomatoes. The HR-MAS NMR spectra of the tomato pulp of three different taste varieties were investigated during the ripening using chemometrics. Levels of citric and malic acid, carbohydrates and γ-aminobutyric acid (GABA) could be used to differentiate the three varieties, and GABA was identified to be the strongest biomarker for the ripening across the three varieties (Pérez et al. 2011). The relative levels of organic acids and carbohydrates during ripening were variety-dependent, and hence, could potentially be used as variety markers.

For assimilation of carbon in plant materials and for the perception of sweetness of fruits, free sugars in plants are essential building blocks. Absolute quantification of sucrose, glucose and fructose in intact melon mesocarp tissue was performed by ^{1}H HR-MAS qNMR by Delgado-Goñi et al. (2013). The fact that less than 30 min is required in total for the analysis together with the high precision of the method makes HR-MAS qNMR appealing over other analytical techniques.

Global metabolite profiling of cultivars of rice (*Oryza sativa* L.) has been performed by Song et al. (2016) using ^{1}H HR-MAS NMR of powdered rice grains without prior extraction. Clear differences between waxy and non-waxy rice cultivars could be observed (fatty acid region of the spectrum) and also differences in the alanine content were observed, which is an indicated of improved resistance to hypoxic stress conditions. Furthermore, the soil and environmental differences were of less importance in the classification than were the cultivars.

A comprehensive ^{1}H HR-MAS study has been conducted to differentiate apple cultivars based on analysis of intact apple tissue (Vermathen et al. 2011). Chemometrics enabled the differentiation of the three cultivars Golden Delicious, Rubens and, Braeburn, which could be ascribed to cultivar-specific variations in the content of acetaldehyde and fructose and to some extent also to in chlorogenic acid, ethanol and lipids. A comparison of the HR-MAS NMR spectra obtained from intact apple tissue and liquid-state NMR spectra obtained from apple juice showed distinct differences in some metabolites, and it was concluded that the HR-MAS technique has the advantage that it provides insight into the native mostly unaffected chemical components. Vermathen et al. (2011) observed differences between aldehydes (–CHO), ethanol and branched amino acids (leucine, valine) in three different apple cultivars (Fig. 1).

A Brazilian study performed by Choze et al. (2013) demonstrated that ^{1}H HR-MAS NMR in combination with multivariate data analysis could separate wild-type cultivars of common bean (Olathe Pinto, Pérola and BRS Pontal) from transgenic lines. The transgenic common bean genotypes studied had higher concentrations of flavonoid compounds than their wild-type counterparts. The study also showed that beans

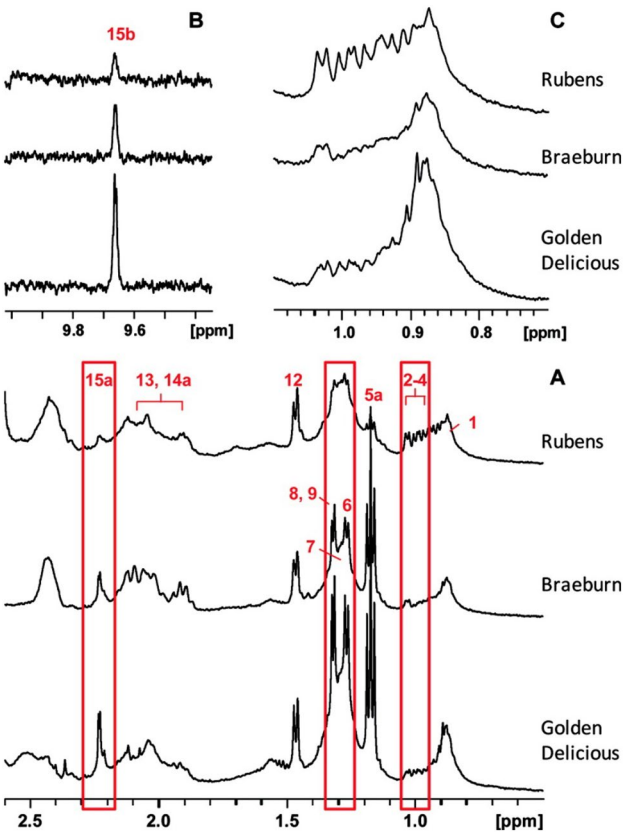

Fig. 1 Comparison of ^{1}H HR-MAS NMR spectra from Golden Delicious, Braeburn, and Rubens cultivars. **a** 0–2.5 ppm. Spectral regions, which contribute to discrimination of cultivars are marked with a red box. **b** 9.35–10 ppm, AeCHO peak. **c** 0.7–1.1 ppm, Leu, Ile,Val peaks. Reprinted with permission from Vermathen et al. (2011). Copyright (2011) American Chemical Society. (Color figure online)

of the same cultivar grown under green-house conditions could not be separated from beans grown on fields, thereby providing evidence that based on their metabolite profile, greenhouse-grown beans and beans grown on fields can be expected to have the same nutritional value.

The geographical authentication by metabolite profiling using HR-MAS NMR and chemometrics for typical foods consumed in the Mediterranean diet has been shown possible by Corsaro et al. (2015). For traditional Italian food products like cherry tomatoes of Pachino, Interdonato lemon of Messina, extra virgin oils of Sicily and red garlic of Nubia, all protected by so-called PGO/PGI/PAT certificates, metabolites were identified that could potentially be used for authentication.

3 MAS NMR: from seeds to bread

MAS NMR techniques are useful in the study of starch, and MAS NMR techniques find applications on both seeds, starch granules and have also been applied to study the

dynamics of the bread making process. MAS NMR studies on starch are typically involve solid-state MAS techniques and ^{13}C CP MAS is a common experiment. An overview of the studies is listed in Table 2, and the following sections provide a description of research conducted with MAS NMR techniques in this area.

3.1 Carbon assimilation in plant seeds

Monitoring changes in carbon assimilation profiles by ^{1}H HR-MAS NMR during plant growth is illustrated elegantly in work by Seefeldt and coworkers (Seefeldt et al. 2008). In this study the grain-filling process of barley was monitored at 9–47 days after flowering (DAF) in three beta-glucan mutants. The detection of a number of multiplets and sharp resonance peaks in the HR-MAS NMR spectra indicated the presence of numerous smaller, mobile molecules at day 9. Free glucose, amino acids, peptides, protein and free fatty acids were most abundant in the early phase, while in the later stages of the grain filling (day 23, 47) lipid, starch, amylopectin and β-glucan signals were dominating. An important observation using the HR-MAS NMR technique in the study of cereals is that the starch crystallinity causes a major part of the starch to be invisible, while the more mobile plant lipids are dominating in the HR-MAS NMR spectrum (Fig. S2). Furthermore, a short review on the use of MAS NMR to study plant seeds including assignments of major substance classes is given by Bardet et al. (2006).

3.2 Starch and starch heterogeneity

Starch is the main energy storage component in higher plants organized in granules and is the most studied component of food science using solid-state MAS NMR. In the twentieth century the complexity of starch organizational structure, polymorphs and crystallinity were primarily investigated using microscopy and X-ray crystallography. Starch is a polysaccharide existing as linear α-1,4-linked amylose mixed with amylopectin, which is α-1,6-branched molecule with shorter chains of α-1,4-linear modules. Figure 2 depicts the organizational overview of starch granula as illustrated by Tang and Wang (2006).

With the availability of MAS NMR, both the starch crystalline forms as well as the amorphous counterparts can be investigated and their ratios and variability between species can be determined (Gidley and Bociek 1985, 1989). Several early MAS NMR studies correlated the different crystal forms of starch with the known X-ray data and explored the amorphous regions (Gidley and Bociek 1985; Paris et al. 2001a, b; Tang and Wang 2006; Tan et al. 2007). With this knowledge, processes like gelatinization, cross-linking and hydration properties of different starch types have been studied intensively. Also processing of starch through modifications and enzymatic treatments has been investigated (Gidley 1989; Paris et al. 1999; Tang and Hills 2003; Larsen et al. 2013). Food processing of starch from dough to bread making and finally bread staling have also been characterized (Brescia and Sacco 2006; Calucci and Geppi 2006; Mihhalevski et al. 2012).

The magnetization transfer of ^{13}C CP MAS NMR experiments intensifies the resonances from the more immobile and crystalline parts of the starch (A, B types, both double helices) and V-type (single helix) polymorphs), while the ^{13}C SP experiment quantitatively reports on both the granule layers of mobile amorphous starch and the immobile crystalline parts. For quantitative results, it is important to work with these types of samples at known water activity levels for comparison and reproducible results.

Cereal non-waxy starch granules have been investigated by Morgan et al. (1995). Besides detection of branching in amorphous non-waxy starch using SP MAS NMR, Morgan et al. (1995) showed that amylose/lyso-phospholipid inclusion complexes are located separately from other crystalline parts. The challenge of starch heterogeneity was further investigated by Paris and co-workers (Paris et al. 1999, 2001a, b). Different crystalline and amorphous forms of starch were isolated and employed to simulate the spectra for more native samples. Extruded potato starch was used as reference for amorphous material, and spectral subtraction was employed for spectra obtained from native starch followed by Gaussian deconvolution to investigate the composition of native potato and native waxy maize. The principles of Gaussian deconvolution, which is often needed for complex mixture decomposition in MAS NMR spectra, are illustrated in Fig. S3. WISE (wide line separation) is an experiment based on the use of cross-polarization to characterize molecular dynamics (Schmidt-Rohr et al. 1992), and Paris and co-workers (Paris et al. 2001a, b) also demonstrated how the 2D WISE experiment and contact time MAS NMR experiments can be applied to elucidate complex structures (4–5 amorphous types dependent on plant source) and the interactions between starch and water.

Tan and co-workers (Tan et al. 2007) were able to quantify the relative amounts of amorphous, single helix and double-helical components in starch granules and found that native starches with high amounts of amorphous components also contained higher levels of the single helix V-type.

3.3 Hydration and the bread making process

Starch is the main storage component of plants, and the build-up of starch in grains as well as the subsequent modification via flours, gluten or doughs have been studied using MAS NMR (Brescia and Sacco 2006; Calucci and Geppi 2006). Hydration properties and enzymatic modifications during gelatinization were investigated by Larsen

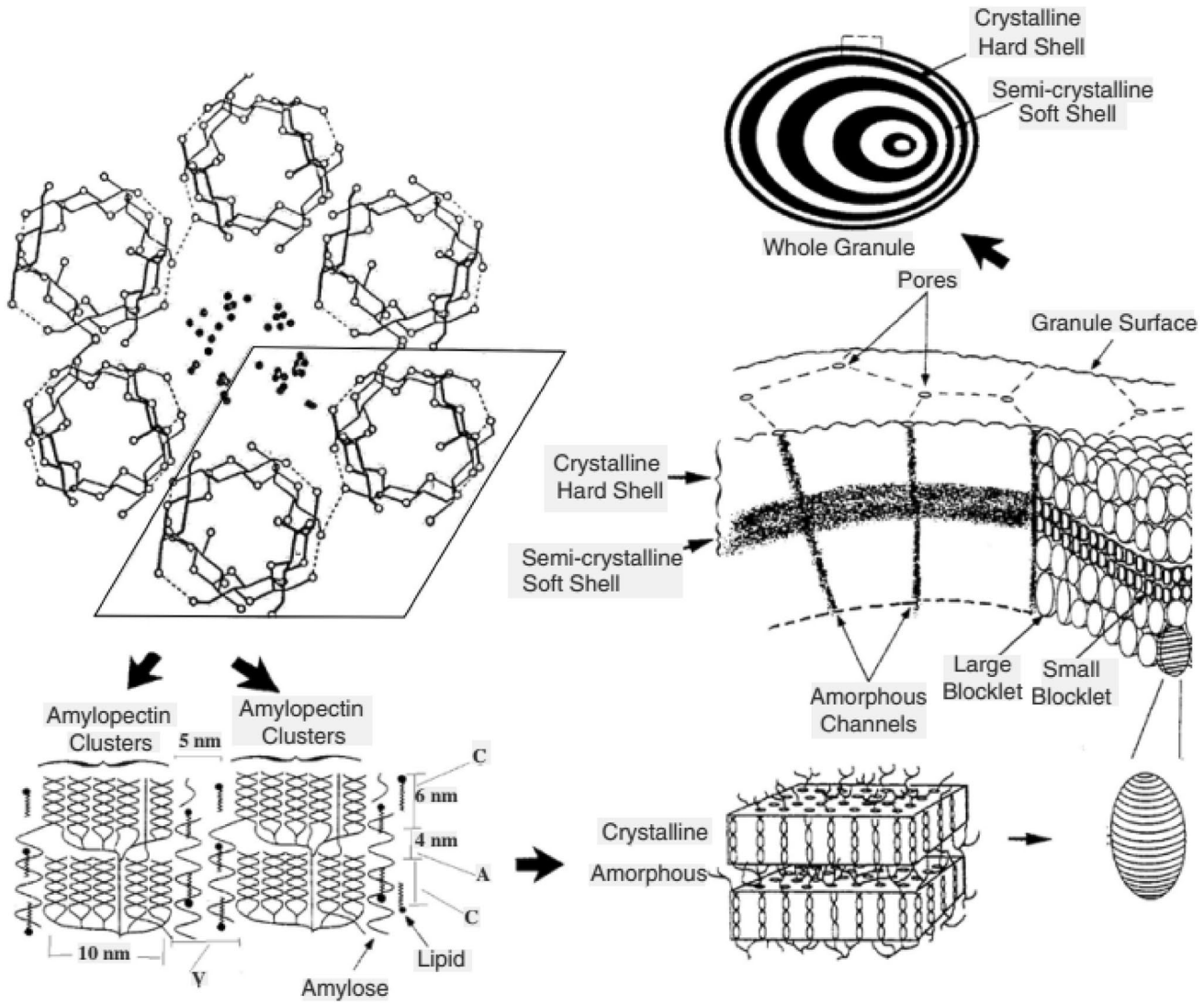

Fig. 2 Schematic presentation of the structural features of the native starch granules. Reprinted from Tang and Wang (2006) with permission from Springer

et al. (2013) in wheat, potato and waxy maize type of starches. Gelatinization and enzymatic trimming of side-chains of the potato starch led to a higher degree of order for starch in suspension and the ordering of the 1 → 6 branching linkages required a hydration level of around 70%. Using ^{31}P SP and ^{31}P CP MAS NMR experiments it was shown that the presence of ^{31}P in the immobile starch only was observed in the native non-processed starch. After gelatinization, the ^{31}P signals revealed mobility and disordering even after drying. The ^{31}P NMR signals may originate from either phosphate- monoesters on starch (potato) or from phospholipids (mainly Lyso-PC) enclosed in starch granules of cereals (wheat, corn, oat, rice). Utilization of differences in the $T_{1\rho}$ relaxation between different starch types with the PRISE (proton relaxation induced spectral-editing) technique enabled Tang and Hills (2003) to differentiate native starch types in pea, corn and potato and to illustrate their hydration dynamics.

Structural changes during the bread-making process have also been investigated (Mihhalevski et al. 2012) using ^{13}C CP MAS NMR combined with spectral deconvolution. In Fig. 3b changes in the crystalline structure were observed as a decrease in peaks B, C and D (double helices) during baking from rye flour to bread. During the stalling process (rye bread 0 h to 11 days) a crystalline structure is formed, observed as increase in peaks B, D and E (Fig. 3a). Furthermore, differences in the starch retrogradation between rye bread from sourdough and yeast-raised wheat bread could be identified as the formed crystalline structure having less of the D and more of the B component in the ^{13}C CP MAS spectra of wheat bread than rye bread (Fig. 3a). In studies of starch component changes in the process from seed to bread,

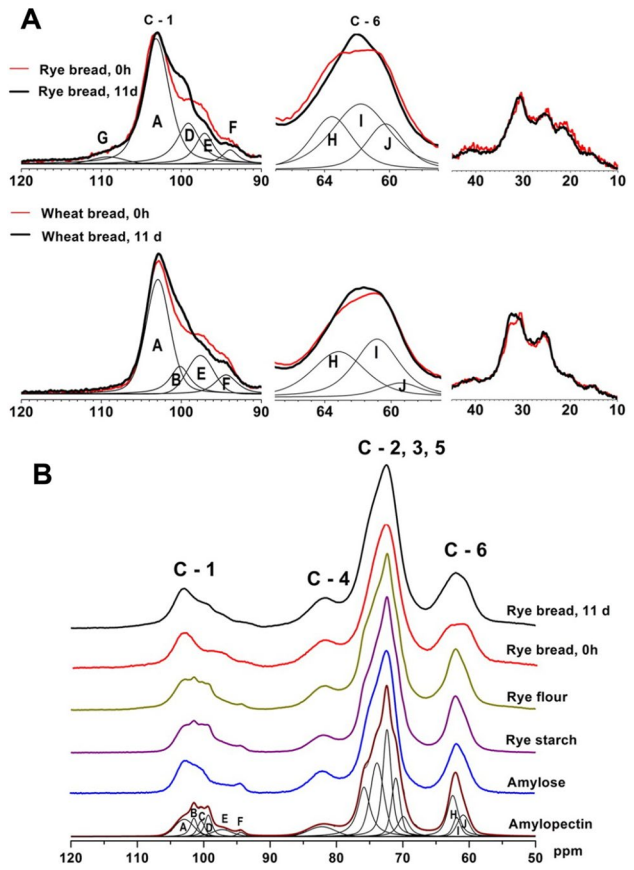

Fig. 3 ^{13}C CP MAS NMR spectra of rye and wheat bread (**a**) and amylopectin with decomposition of resonances, amylose, rye starch, flour, and bread (**b**). Interpretation of the individual components: C1-A (102.9–103.2 ppm), amorphous region of amylopectin (branching points) and V-type single helix; C1-B (101.4–101.5 ppm), double helices; C1-C (100.1–100.5 ppm), double helices; C1- D (99.1–99.8 ppm), double helices; C1-E (96.4–97.7 ppm), glucose units near α-(1 → 6) linkages within the branched regions; C1-F (93.8–94.7 ppm), associated with constrained linkages; C1-G (106.9–109.1 ppm), C1 of rye cellulose. Reprinted with permission from Mihhalevski et al. (2012). Copyright (2012) American Chemical Society

the MAS NMR measurements are often supported by X-ray diffraction (XRD), differential scanning calorimetry (DSC) and liquid-state NMR.

4 MAS NMR in meat and muscle-based foods

Since ancient time, meat and muscle-based foods have been an important part of our diet. Meat and muscle-based foods also belong to the more expensive food categories, which probably has contributed to the fact that many NMR applications have been initiated and established on such products over the last 2–3 decades.

4.1 Muscle-based foods

4.1.1 Post mortem metabolism

After slaughtering a complex process follows where muscle tissue is converted into meat. The processes taking place during the conversion of muscle to meat are decisive for the final meat quality and involve post mortem metabolism. The post mortem metabolism is a dynamic process initiated by the disruption of the blood supply to the muscles leading to anaerobic conditions while biochemical processes in the muscle are still active. Due to the dynamic character of the post mortem process, measurements that enable a time course to be followed are attractive. Bertram et al. (2004a) were the first to apply a ^{1}H HR-MAS NMR methodology to study the post mortem metabolism of rabbit muscles dynamically over a 24 h period. In order to introduce variations in the post mortem metabolism the study included muscle samples excised post mortem from both un-treated rabbits and rabbits injected with adrenaline 4 h before sacrifice to deplete muscle glycogen stores. In relation to meat quality, an important parameter in the post mortem course is the pH decline taking place as a result of lactate formation from degradation of muscle glycogen stores. In the study by Bertram et al. (2004a) lactate formation in the muscle was quantified from the ^{1}H MAS NMR spectra by addition of exogenous lactate and it was demonstrated that ^{1}H MAS NMR spectroscopy can provide detailed and quantitative information on the progress in the post mortem lactate formation. An attractive feature of ^{1}H NMR spectroscopy is the fact that the chemical shift of some metabolites is pH-dependent, which can be used to determine pH of a sample. Based on the chemical shift of carnosine, Bertram et al. (2004a) also calculated pH from the ^{1}H HR MAS NMR spectra acquired on the rabbit muscle samples. The correlation between lactate formation and muscle pH was found to be high, which reflects the capability of the ^{1}H HR MAS NMR spectroscopy to determine these two parameters of importance for meat quality. The study by Bertram et al. (2004a) was in fact multinuclear; while performing MAS, both ^{1}H NMR and ^{31}P NMR spectra were acquired successively on the rabbit muscle samples. The ^{31}P NMR spectra enabled the detection of the energy-rich phosphorus compounds in the muscle including ATP, ADP, and phosphocreatine and their post mortem degradation course could be followed (Bertram et al. 2004a). No significant differences were observed in the post mortem degradation of phosphocreatine between glycogen-depleted and control muscles. It therefore seemed plausible to conclude that ^{1}H HR MAS NMR spectroscopy is more attractive for studying parameters of importance for water-holding capacity than ^{31}P MAS NMR spectroscopy.

Applications of ^{13}C cross-polarization MAS NMR spectroscopy have also been reported on post mortem muscles.

However, in contrast to ^{1}H and ^{31}P MAS NMR studies on post mortem muscle, these ^{13}C CP MAS NMR studies have been conducted on frozen muscle samples to inhibit enzyme activities and thereby stop the post mortem processes. Already in 1993 Quistorff et al. demonstrated how ^{13}C cross-polarization MAS NMR spectroscopy can be applied on muscle tissue to study muscle glycogen. Bertram et al. (2003) expanded this work on use of ^{13}C cross-polarization MAS NMR spectroscopy on muscle tissue and applied it on muscle biopsies of *m. longissimus dorsi* taken at various time points during the post mortem period from pigs exposed to different degree of pre-slaughter stress. Glycogen content of the muscles was quantified from the ^{13}C CP MAS NMR spectra and correlation to biochemical determinations of glycogen established that ^{13}C CP MAS NMR spectra can provide quantitative data on muscle glycogen content. Intriguingly, the ^{13}C CP MAS NMR spectroscopic study by Bertram et al. (2003) also revealed variations in the methylene lipid signal at ~35 ppm that seemed to be associated with pre-slaughter stress conditions, and it was hypothesized that this finding was reflecting a stress-induced disintegration of cell membrane lipids in the muscle cells. In a later study, a relation between the ^{13}C CP MAS NMR spectra and water mobility and distribution in the muscles was indicated (Bertram et al. 2004b), which has contributed to elaboration on a biophysical model explaining the complex post mortem processes (Bertram et al. 2004c).

4.1.2 Meat

As pioneering work, Brescia et al. (2002) reported the first ^{1}H HR MAS spectrum of meat. In this study, beef meat was minced and freeze-dried, subsequently resuspended in D_2O and HR-MAS measurements were carried out using a 400 MHz spectrometer at spinning speed of 4.5 KHz. Thus, Brescia et al. (2002) demonstrated that ^{1}H HR-MAS enabled the detection of a number of metabolites in meat using a relatively simple approach. Two-dimensional spectra were also reported, and the metabolites assigned in this work included alanine, valine, glutamine, tyrosine, carnosine, creatine, lactate and glucose (Brescia et al. 2002). Italian researchers with Brescia and co-workers included have subsequently elaborated on the use of ^{1}H HR-MAS on meat and focused on using the technique as a tool for characterizing lamb and goat meat (Sacco et al. 2005; Longobardi et al. 2012). Authentication of geographical origin is an area of interest to receive EU's protected designations of geographical indications (PGIs), and therefore Sacco et al. (2005) investigated the potential of ^{1}H HR-MAS measurements as one out of several analyses to discriminate lamb meat samples from 4 different breeds and originating from three different locations of southern Italy. The study only included 25 meat samples and therefore the study design lacked power to draw any conclusions on the potential of ^{1}H HR-MAS to discriminate lamb meat according to geographical origin (Sacco et al. 2005). However, Sacco et al. (2005) claimed that the HR-MAS NMR data combined with stable isotope ratio determination would be a superior method for origin determination as compared to conventional methods. Differentiation of geographical origin by ^{1}H HR-MAS has also been investigated on processed meat. Metabolite profiles of 23 dried meat samples, resembling Bresaola, which is an Italian dried beef product that has been aged for two to three months, from five different countries (USA, Switzerland, Canada, Brazil, Australia) were obtained by ^{1}H HR-MAS (Shintu et al. 2007). Both PCA and super-vised multivariate methodologies revealed differentiation of the samples that mainly could be attributed to variations in fat content and to some extent to the amount of free amino acids present in the meat (Shintu et al. 2007). Variations in fat content can easily be introduced from variations in raw materials and variations in the meat content of amount of free amino acids will be dependent on the extent of proteolysis, which will proceed over time. Therefore, the limited amount of samples included in the study did not allow substantiating the potential of the technique for determination of geographical origin. On beef, Ritota et al. (2012) investigated the potential of ^{1}H HR-MAS to discriminate meat from two different muscles (*longissimus dorsi* and *semitendinosus*) and found the discriminant classification power being good for some but not all breeds.Recently,^{1}H HR-MAS has also been employed to study manufacturing of dry-fermented sausages (Garcia-Garcia et al. 2018). This work reported comprehensive spectral assignment and therefore provides a very good indication on the potential that ^{1}H HR-MAS holds in terms of the range of metabolites that are detectable on meat as a sample matrix (Garcia-Garcia et al. 2018).

4.2 Fish and seafood

MAS NMR studies of fish have mainly focused on lipid characterization and metabolite profiling, which will be covered in the following sections.

4.2.1 Lipid composition

Fish is in general considered a healthy food item due to the fact that fish is considered an important dietary source of unsaturated fat, especially n-3 and n-6 fatty acids. As a consequence of the fact that fish lipids play a crucial role for the nutritional value of the fish, methodologies for analysis and characterization of the lipid profile of fish are essential. Several studies have explored the potential of ^{1}H HR-MAS NMR spectroscopy to characterize the composition of intact fish tissue, and Table 2 lists ^{1}H HR-MAS NMR spectroscopic studies reported on various fish species. Salmon is

the most frequent fish species studied with ^{1}H HR-MAS NMR. Most ^{1}H HR-MAS studies on fish have focused on the quantitation of total amount of n-3 fatty acids. Furthermore, the ^{1}H MAS studies on fish have especially emphasized the detection and quantification of the specific n − 3 fatty acids fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are considered to possess beneficial effects in relation to prevention of cardiovascular diseases (Kromhaut and Goede 2014) but also on brain and nerve system (Riediger et al. 2009). Studies have suggested that EPA may be efficacious in treating depression and schizophrenia (Song and Zhao 2007; Martins 2009).

Both Nestor et al. (2010) and Bankefors et al. (2011) described how the quantification of the fatty acids can be achieved from diffusion-edited ^{1}H HR-MAS NMR spectra of fish. The ^{1}H signal from the methyl group in the n-3 fatty acids (approx. 0.93 ppm) can be distinguished from ^{1}H signal from the methyl group of other fatty acids (approx. 0.83 ppm), and thereby the percentage n − 3 fatty acids can be determined. For quantification of the relative amount of DHA, a resonance from the =CHC**H**$_2$–C**H**$_2$–COOR methylene groups (approx. 2.35 ppm) can be used and expressed relatively to total fatty acids content by using the resonance from the –C**H**$_2$–COOR (approx. 2.25 ppm). For quantification of the relative amount of EPA, the –C**H**$_2$–CH$_2$–COOR of EPA (approx. 1.65 ppm) is shifted downfield thereby can be distinguished from the corresponding resonance from other fatty acids. Comparisons of determinations of the relative amounts of n − 3 fatty acids, DHA and EPA by ^{1}H HR-MAS NMR spectroscopy and determinations obtained by GC analyses have shown that GC yields slightly higher levels of n-3 fatty acids than ^{1}H HR-MAS NMR spectroscopy (Aursand et al. 2008; Nestor et al. 2010). This can likely be ascribed to the extraction step done prior to GC analyses, which may result in changes in the fatty acid composition, highlighting the necessity of methods such as ^{1}H HR-MAS NMR, which enables measurement directly on the unmodified food matrix, and thereby provides the most accurate determination.

4.2.2 Metabolites in fish

Castejon et al. (2010) reported the most comprehensive spectral assignment work done on ^{1}H HR-MAS NMR data obtained on fish. A total of 160 resonances were detected, and out of these 152 resonances were assigned to approximately 40 different metabolites representing carbohydrates, nucleoside derivates, osmolytes, amino acids, dipeptides, organic acids, fatty acids and lipids. Freshness is of crucial importance for fish quality. Several methods to evaluate and determine fish freshness have been proposed. Three chemical methods have been widely adopted; (i) determination of total volatile bases nitrogen, (ii) determination of trimethylamine (TMA) nitrogen, and (iii) determination of the so-called K-value, which expresses the degree and extension of ATP degradation into inosine and hypoxanthine. Recently Heude et al. (2015) showed the capability of ^{1}H HR-MAS NMR of intact fish muscle to determine TMA nitrogen and to determine the K value of fish. No direct comparison with reference methods was carried out in the study, but determination of the K value during storage and comparison with values reported in literature showed agreement between the K value determined by ^{1}H HR-MAS NMR and the K value determined classically with HPLC analyses (Heude et al. 2015). For determination of TMA nitrogen using the integral of the TMA peak at 2.89 ppm in the ^{1}H MAS NMR spectrum, consistency with TMA nitrogen values reported in literature was likewise obtained (Heude et al. 2015). Consequently, it was demonstrated that ^{1}H HR-MAS NMR has a potential as a fast method to determine freshness of fish samples as the method does not require any sample extraction steps.

The use of ^{1}H HR-MAS NMR to elucidate processing-induced changes in fish muscle is relatively sparse. Castejon et al. (2010) investigated changes in the metabolite composition of smoked salmon through a 45-days storage period by using ^{1}H HR-MAS NMR spectroscopy. The study demonstrated that during storage of smoked salmon, a degradation of carbohydrates and nucleotides is taking place (Castejon et al. 2010), implying that ^{1}H HR-MAS NMR may provide information useful to evaluate freshness of stored fish. Irradiation of foods is a tool to control pathogenic microorganisms and thereby ensure food safety. Villa et al. (2013) reported an investigation on the use of ^{1}H MAS NMR spectroscopy to elucidate the impact of irradiation on the metabolite profile of smoked salmon. In addition to a control consisting of no irradiation, two levels of irradiation dose were included; 1 and 4 kGy, respectively. Visual inspection of the ^{1}H HR-MAS NMR spectra obtained did not reveal any pronounced effects of irradiation on the metabolite profile of smoked salmon, however, from PCA it was possible to disclose that the intensity of some metabolites was slightly altered upon irradiation. These metabolites included creatine, cholines, trimethylamine oxide (TMAO), taurine, lactic acid, sucrose, inosine, anserine and triglycerides. Villa et al. (2013) suggested the use of the specific metabolites TMAO, creatine and sum of phosphorylcholine and glycerophosphorylcholine for discrimination of non-irradiated and irradiated fish. The findings are interesting and promising, but further studies should be pursued to validate the findings and ensure that these metabolites are not susceptible to variations caused by other factors as such raw material variations and factors related to processing.

5 MAS NMR in cheese and dairy products

Many cheeses and dairy products are semi-solids and the potential of ^{1}H HR-MAS for product characterization of cheese and dairy products is easily envisaged from a biophysical point of view. Furthermore, as protected designation of origin (PDO) as well as long-term ripened cheese products exist on the market, cheese and dairy products also represent a food category with a value that makes more advanced analytical measurements feasible. Finally, the quality of many cheese and dairy products are related to the characteristics of both the intrinsic lipids and as well as hydrophilic compounds, which makes ^{1}H HR-MAS applications attractive as it enables to study both lipids and hydrophilic compounds simultaneously in a 'true' intrinsic food matrix as an extraction step is not needed. Table 2 provides an overview of various MAS NMR spectroscopic studies reported on cheese and dairy products.

5.1 ^{1}H HR-MAS studies

5.1.1 Parmigiano Reggiano cheese

Shintu and co-workers were among the first to develop a ^{1}H HR-MAS spectroscopic methodology for analysis of cheese, and in 2004 they reported a ^{1}H HR-MAS study conducted on Parmigiano Reggiano cheese. Parmigiano Reggiano is an Italian hard cheese that is named after its geographical producing areas in Italy. According to Italian law, only cheese produced in specific provinces may be labelled "Parmigiano-Reggiano", and European law classifies the name, as well as the translation "Parmesan", as a PDO. Later, Shintu and Caldarelli (2005) applied ^{1}H HR-MAS spectroscopy to study Parmigiano Reggiano cheese ripened for 4, 8, 12, 18 and 24 months, respectively. PCA of the ^{1}H HR-MAS spectra obtained could clearly distinguish the cheeses according to duration of ripening. Analysis of the specific spectral differences revealed that alterations in some amino acids and other low-molecular-weight metabolites including serine, methionine, valine, tyrosine, threonine, asparagine, aspartic acid and citrulline characterized the ripening process (Shintu and Caldarelli 2005). The results thereby support that the unique sensory properties of Parmigiano Reggiano cheese can be ascribed to a ripening-induced formation of free amino acids and lipolysis, which gives rise to the distinct taste that characterizes this cheese product.

5.1.2 Emmentaler cheese

Shintu and Caldarelli (2006) have also applied ^{1}H HR-MAS to study Emmentaler cheese. This study focused on geographical origin and Emmentaler cheeses from seven different regions in Austria, Finland, France, Germany and Switzerland were included in the study. The ^{1}H MAS analyses enabled to characterize the low-molecular-weight metabolite profile of the Emmentaler cheeses directly using 20 mg sample sizes and thereby validated the potential of ^{1}H HR-MAS analyses for a direct analysis of the cheese without any extraction steps that may alter the properties of the product. The cheese samples included in the study originated from cheese made of both raw and pasteurized milk, and canonical analysis of the data obtained indicated spectral differences between cheeses made of raw and pasteurized milk, respectively. However, the relatively small number of samples included in the study and the fact that samples originated from cheese ripening for varying time periods means that further studies are needed to confirm the findings reported by Shintu and Caldarelli (2006).

5.1.3 'Mozzarella di Bufala Campana' (MBC)

'Mozzarella di Bufala Campana' is an Italian cheese that received the PDO trademark in 1996. Mazzei and Piccolo (2012) studied the metabolite profile of MBC cheese samples by using ^{1}H HR-MAS NMR spectroscopy. Three different pulse sequences were applied; a standard 1D spectrum, a diffusion-edited spectrum and a CPMG-edited spectrum. While a diffusion-edited spectrum enhances signals from lipids, the CPMG-edited spectrum enhances signals from low-molecular weight metabolites, and by employing both pulse sequences Mazzei et al. (2012) focused on both the lipid profile of the cheese as well as the low-molecular weight metabolites present in the cheese. The study included 37 samples supplied directly from a dairy factory and thereby of known origin and 12 commercial MBC samples with PDO certification obtained from a local dairy market and the study focused on using ^{1}H HR-MAS NMR spectroscopy to discriminate between the two classes of samples. Both PLS-DA and HCA gave 100% total correct classification for ^{1}H HR-MAS spectra obtained with the CPMG pulse sequence.

5.1.4 Soft cheese

^{1}H HR-MAS NMR spectroscopic studies have also been conducted on soft cheeses. Lamanna et al. (2008) employed ^{1}H HR-MAS NMR spectroscopy to acquire standard 1D spectra of soft cheese and found that mainly lipid signals could be detected. Lamanna et al. (2008) also investigated effect of packaging and storage time and found that no changes could be observed in the lipid signals detected in the ^{1}H HR-MAS NMR spectra. Lamichhane et al. (2015) studied the effects of rennet, starter culture and duration of salting time on the metabolite profile of soft cheeses by using ^{1}H

HR-MAS NMR spectroscopy. In contrast to Lamanna et al. (2008), Lamichhane et al. (2015) employed CPMG-edited spectra, which enabled the detection of 20 low-molecular metabolites, and Lamichhane et al. (2015) were able to identify effects of salting time on content of lactate in the cheese. Furthermore, Lamichhane et al. (2015) also coupled the metabolite profiles of the soft cheese obtained by ^{1}H HR-MAS NMR spectroscopy with results obtained from sensory analyses of the same cheeses. These correlation analyses demonstrated a relation between the cheese metabolite profiles and the sensory attributes butter milk aroma ($r^2 = 0.55$) and butter aroma ($r^2 = 0.52$), and further exploration of the correlations revealed that they could be attributed to the metabolite acetoin (Fig. 4). Consequently, Lamichhane et al. (2015) demonstrated that ^{1}H HR-MAS NMR spectroscopy is a useful technique to analyse cheese metabolite characteristics as function of processing conditions and to identify distinct cheese metabolites affecting the sensory properties.

5.2 Milk protein concentrate

Globally there is an increasing demand for milk products, and especially in Asia there has been a long-term increase in per-capita milk consumption. As a consequence, still increasing volumes of dairy products are produced as dehydrated powders to reduce transport loads and increase shelf life. Haque et al. (2015) employed solid-state ^{13}C NMR CP MAS to study physico-chemical changes during up to 14 weeks storage of milk protein concentrates. Intriguingly, it was found that a shorter contact time was required for magnetization build-up in non-aged samples compared with stored samples, which was interpreted as a reduction in rigidity of molecular domains as a result of interactions with water during storage at high humidity (Haque et al. 2015). In this way, the study provided useful information that may assist in understanding the molecular mechanisms involved in a reduction in solubility of milk powders during long-term storage.

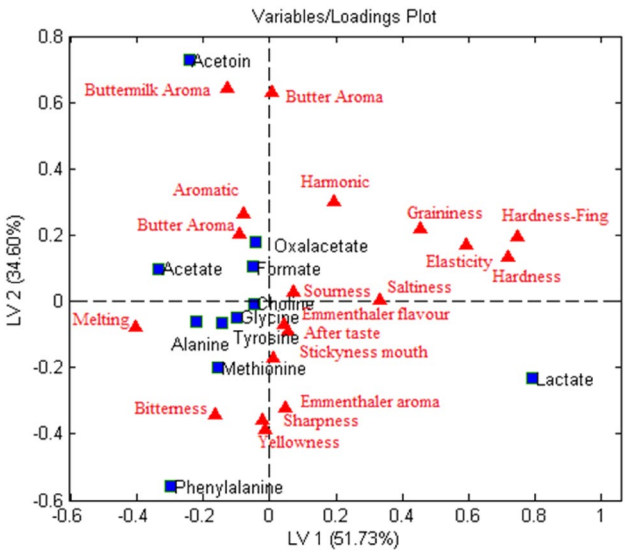

Fig. 4 Partial least squares (PLS) loading plot illustrating the means of different sensory profiles and selected metabolites in soft cheese. Blue squares represent metabolites determined by ^{1}H HR-MAS and red triangles represent sensory attributes. Reprinted from Lamichhane et al. (2015) with permission from The Royal Society of Chemistry. (Color figure online)

5.3 ^{31}P MAS NMR spectroscopy

While the majority of MAS NMR spectroscopic analyses are based on ^{1}H, few studies have also employed ^{31}P MAS NMR spectroscopy on cheese. Interestingly, Gobet et al. (2010) employed single pulse ^{31}P MAS NMR spectroscopy and ^{31}P CP MAS NMR spectroscopy on soft cheese samples to study the distribution of phosphorus metabolites. The SP ^{31}P MAS NMR spectra obtained revealed the presence of two major peaks. Gobet et al. (2010) assigned one of these peaks (approx. 0 ppm) to dissociated Pi, and the peak represented about 13% of the total phosphorus content in the cheeses. After deconvolution of the other peak (approx. 0.5–1.2 ppm), two fractions were identified that were assigned to mobile phosphate molecules in the soluble phase and phosphate molecules present in casein micelles that are less mobile because of the constraints imposed by the colloid nature of the casein micelle. A CP MAS experiment can be targeted at the mobility of the molecules by adjusting the Hartmann–Hahn contact time. When conducting ^{31}P CP MAS experiments with a short contact time on the cheeses to attenuate mobile phosphorous, Gobet et al. (2010) detected only one peak, and thereby the ^{31}P CP MAS experiments could confirm the assignment of the two fractions identified in the SP ^{31}P NMR experiment. Gobet et al. (2010) also investigated how the distribution of phosphorus was affected by the NaCl content of the cheese by comparing ^{31}P MAS NMR spectra obtained on cheeses with two levels of NaCl. Intriguingly, this study showed that the ratio between mobile and immobile phosphate was affected by NaCl, which was explained by an effect of NaCl on the Ca^{2+} concentrations in the diffusible phase and thereby also on the phosphorus molecules in the colloidal phase.

6 Taste, sensory and texture

The pleasure and enjoyment of food intake is strongly related to taste, sensory and texture properties of the food item and the perception of the physical and chemical attributes of the food. Crystallinity, viscosity, gel-strength and lubricity are physical parameters that are determined by complex interactions between water, lipids, proteins, polysaccharides and

other food ingredients. For measurement of these parameters, food science disciplines like rheology, tribology calorimetry and low-field NMR relaxometry are important techniques, but MAS NMR spectroscopy can also contribute with a unique physical/chemical characterization of food systems. Most often a combination of different analytical techniques is needed to thoroughly understand the behavior of food systems. An example is the study of hydration and behavior during heating of doughs. Lopes-da-Silva et al. (2007) employed a combination of oscillatory rheology, shear viscometry and ^{1}H MAS NMR spectroscopy to study molecular mobility and water-protein interactions in two types of undeveloped wheat doughs. ^{1}H MAS NMR spectroscopy revealed that the hard variety dough was characterized by faster protein-water interactions. By combining the various analyses, a molecular model explaining differences in the two types of wheat doughs representing a soft and hard variety dough, respectively, was proposed (Lopes-da-Silva et al. 2007).

6.1 Food ingredients decisive for food function and texture

In addition to starch, cellulose and hemicellulose are important structural building blocks for plant-based foods. Due to the insoluble nature of these biopolymers, the use of XRD and MAS NMR are obvious and relevant analytical choices. Many of the early studies on the cellulose crystallinity originate from wood/pulp research (Larsson et al. 1999). Due to an increased interest in the enzymatic degradation of biomass to energy sources, e.g. bio-ethanol, the MAS NMR technique has regained attention in order to study and understand cellulose degradation. An example is the study by Park et al. (2010) who compared the crystallinity index (CI) determined by X-ray diffractograms and MAS NMR. The study concluded that the CI determination is method-dependent and it was questioned if CI can be used to predict enzyme digestibility.

Microcrystalline cellulose and modified cellulose are used as bulking agents and thickening agents in both the cosmetic and food industry, respectively. Carboxy-methylated cellulose (CMC) is a more soluble form of cellulose and has extensive usage as stabilizer in food products. Heinze and Koschella (2005) reviewed the use of NMR Spectroscopy including the use of MAS NMR to determine the degree of carboxy-methyl ether substitution on the cellulosic backbone. Another example of MAS NMR characterization of food ingredients is the structural building blocks of homo-polymeric polysaccharide alginate, harvested from brown seaweed. The ratio of mannuronic/guluronic acids can be determined using MAS NMR (Salomonsen et al. 2009). The ratio of mannuronic/guluronic acids is determining if the resulting food gel becomes brittle or softer and elastic.

The structure and interaction of hetero-polymeric polysaccharides like pectin have been investigated by MAS NMR experiments. Synytsya et al. (2003) reviewed the determination of structural features on the homo-galacturonan backbone of the pectin molecule, degree of methylation, and O-acetylation. By peak-fitting, differences between citrus, apple and sugar beet pectin are reported. The structural heterogeneity of side-chains of pectin is well known. Pectin is a part of plant cell walls together with cellulosic components and hydration of the pectin is important for the texture of plant materials. Similar purified pectin is used as gelling agent in food systems. Hydration of the enzymatic modified pectin rhamnogalacturonan sidechains has been studied by MAS NMR of cell wall potato pectin (Larsen et al. 2011). The experiments showed that the arabinan sidechains hydrate faster than galactan sidechains. These MAS NMR studies assist in understanding how altering the pectin structure influences the stabilizing/gelling properties, hence the resulting texture of the food.

6.2 Flavour-related studies

Nature is inherently rich in flavour components and combined with reaction-products from food processing (e.g. Maillard products, bitter peptides), this adds to a cascade of flavour components triggering individual human receptors in the mouth, nose and gut.

Coffee is consumed in large quantities globally and may have impact on human health (Nogueira et al. 2011). The processing of coffee beans from green beans to roasted coffee has been investigated using MAS NMR. Roasting conditions and coffee bean variety were investigated (Ciampa et al. 2010), and MAS NMR was able to demonstrate that process conditions affected the concentrations of several compound classes. In the ^{1}H HR-MAS NMR spectrum of coffee, changes in sugars, amino acids, caffeine and chlorogenic acid could be followed during the roasting process. In addition, products directly derived from the roasting like pyrazines, acrylamides, melanoidins, some of which are Maillard products, could also be monitored by MAS NMR without extraction. In addition, the distribution of chemical components in commercial coffee granules has been investigated (Nogueira et al. 2011). These works show that MAS NMR can be used as a sensor in food quality control as well as in food processing powder development. Another example using MAS NMR to study flavour perception linked to food structure, is demonstrated in the study on the correlations between sensory properties of semi-hard cheese and HR-MAS NMR data (Lamichhane et al. 2015) described in Sect. 5.4. The field of foodomics as defined by Capozzi and Bordini (2013), multidisciplinary studies between MAS NMR and other measures of taste, sensory attributes and texture, represents an emerging field with a very large potential for further understanding of structural changes and

their correlations to perception and to physiological changes due to food choices.

7 Intact tissue NMR in dietary intervention studies

Nutritional sciences are important to understand how food intake influences human health and wellbeing. Nutritional sciences have emerged and developed and now involve both comprehensive studies on direct endogenous metabolic effects, effects induced through complex interactions with the microbiome, and all the way to epigenetics, e.g., how food may alter our genome. One of the tools to understand the nutritional influence is the use of metabolic profiling in animal intervention studies. Solution state NMR is a classical tool in metabolomics of food intervention studies analysing e.g. blood, urine, faeces and organ extracts (Brennan 2014; Manach et al. 2009). This field has been expanded to intact tissue analysis of muscle, liver and other organs using HR-MAS NMR. A protocol from Beckonert et al. (2010) is an excellent practical entry into this field. While HR-MAS studies on intact tissue dealing with pharmaceutical research are numerous, studies focusing on nutrition are sparse. Examples of HR-MAS studies in nutrition research include a study by Bertram et al., who employed ^{1}H HR-MAS NMR to characterize liver tissue from hypercholesterolemic pigs fed either rye or wheat fibre (Bertram et al. 2007). Using multivariate data analysis, it was demonstrated that the liver metabolite profile of hypercholesterolemic pigs fed a high-fibre rye bread differed from that of pigs fed high-fibre wheat bread with respect to both the lipoprotein fractions and the choline-containing compounds (Bertram et al. 2007). These findings revealed that effects of fibre intake on plasma cholesterol partially can be ascribed to effects on liver cholesterol metabolism and thereby provided new evidence for the health-promoting effects of dietary fibres and their mode of action. Using a rat model, Hennebelle et al. (2015) employed ^{1}H HR-MAS NMR spectroscopy to study the impact of energy restriction on the level of different lipid classes (free fatty acids, cholesterol, phospholipids and triglycerides) in liver and skeletal muscle and showed the capability of the technique to elucidate impact of dietary intake on lipid composition in muscle and liver, the latter of importance for the development of steatosis.

8 Perspectives and outlook

8.1 Technical developments

In many food categories including both animal-based food products and fruit and vegetables, MAS NMR spectroscopy has shown great promise for metabolite profiling. MAS NMR is fascinating in its versatile nature; it can be used both for a quantification of metabolites but also to study mobility and molecular interactions through the use of cross-polarization techniques. Since MAS NMR emerged 2–3 decades ago, numerous studies have been reported on various food categories that demonstrate the potential of MAS NMR to study raw material characteristics, elucidate process-induced changes, and possibly be used for authenticity purposes. At the same time, MAS NMR is not yet in a mature phase but more in a developing phase and many challenges and issues need to be further exploited. As many foods are heterogeneous and MAS NMR is based on small sample volumes, future studies examining the reproducibility of the technique are required.

To our knowledge, studies reporting MAS NMR applications on foods are generally all limited to a sample size below 100 samples, and studies including a larger sample size are needed to confirm MAS NMR's applicability for metabolomics studies where a relatively high number of samples are needed to create robust and solid multivariate data models. A common advantage inherent to MAS NMR is that it eliminates a time- and labor-consuming extraction step. In an established and optimized protocol, sample preparation and acquisition of a MAS NMR spectrum can be achieved in approx. 1 h. and manual work is required for packing of the rotor. These facts highlight that there are prospects open for improvements before MAS NMR can be considered a true high-throughput methodology resembling high-throughput methodologies based on liquid-state NMR techniques. Automated probe heads and sample changers and have become commercially available and further attempts to enhance automatization during data collection are reported (Pecher et al. 2017). Both for MAS and liquid-state NMR, developments to improve the quantification of metabolites from spectral data of complex mixtures are essential to enhance efficiency in targeted metabolomics approaches, and work is on-going in this field (Ravanbakhsh et al. 2015; Röhnisch et al. 2018; Gogiashvili et al. 2019).

Alternatives that are not based on MAS NMR techniques may also pave the future way for NMR studies of semi-solid and solid foods without compromising with an extraction step. Cai et al. (2014a) recently presented a Hadamard-encoded intermolecular multiple-quantum coherence (iMQC) technique for analysis of intact fish tissues that were fitted into standard 5-mm NMR tubes without any sample preparation. The iMQC technique represents an alternative to MAS to overcome the magnetic inhomogeneity that is present in semi-solid and solid materials, and even though conventional MAS resulted in higher spectral resolution than the Hadamard-encoded iMQC technique, the approach reported by Cai et al. (2014a) is considered exciting and may inspire new alternative approaches to conduct NMR analyses directly on semi-solid and solid foods.

Sensitivity is a general problem in NMR spectroscopy and due to the complexity of food systems containing mixtures of a multitude of small metabolites and macromolecules, a sensitivity increase is particular important in food science. An interdisciplinary NMR review (Ardenkjaer-Larsen et al, 2015) points toward different sensitivity enhancements including increased field strength - GigaHertz (> 1000 MHz) magnets – and spin-alignment transfer strategies like Dynamic Nuclear Polarization (DNP). The DNP technique typically needs stable radicals for polarization transfer and is a technique to study e.g. fast reactions, like cellular processes. Research is ongoing, building in DNP radicals in amorphous or glassy biomaterials to extend this technique to the solid state. The technique is still in its infancy but has potential to boost the sensitivity several orders of magnitude and allow for e.g. observing binding of inhibitors to proteins in the viral cell membrane (Andreas el al., 2013). In the hands of food scientists, these techniques could lead to in-depth understanding of mechanisms behind antimicrobial actions of beneficial food bacterial cultures and bioactive molecules hence provide new tools fighting spoilage of food by e.g. yeasts and molds.

Another approach to enhance sensitivity is to use ^{13}C labelling of food constituents. Besides increasing the abundance of ^{13}C, this technique could also be used to investigate the fate of food in the digestive system. Labelling natural polysaccharides by growing plants in ^{13}C CO_2 atmosphere chamber (Soong et al. 2014) and isolating e.g. cell wall materials like pectins, the interactions between polysaccharides and proteins as well as the prebiotics effects could be investigated in food matrices.

8.2 Future application areas

The use of MAS NMR in understanding of taste, sensory and texture properties of food is an almost unexploited area. This is despite the fact that MAS NMR evidently can provide valuable information about intrinsic properties of foods that are decisive for taste, sensory and texture properties, and it can be anticipated that MAS NMR in combination with multidisciplinary studies in future will become a useful tool in food psycho-physics. In addition, the use of MAS NMR in food quality control and in development of food manufacturing processing of solid and semi-solid foods is sparse. More basic research in this area could open for more nutritious food and could lead to new developments of industrial processed food with properties mitigating lifestyle diseases.

An obvious application area for MAS NMR is yet unexploited or potential new food sources originating from nature. One example is the investigation of the nutritional composition of the drought-resistant marama bean grown in the Sahara and sub-Sahara region in countries like Botswana, Namibia and South Africa (Holse et al. 2011). Surprisingly the edible bean from the plant did not contain starch or beta-glucans, but only high amounts of proteins, dietary fibres and unsaturated fat. The water-soluble parts had a high content of the amino acid tyrosine. Other examples of studies using MAS NMR to exploit crops include Mutungi et al. (2012) who investigated crystallinity of debranched cassava starch and the work by Cai et al. (2014b) who investigated structure and functionalities of C-type starches from pea seeds, faba beans, water chesnuts and yam rhizomes.

Another area that has increasingly gained interest is the discovery of new food raw material sources from the marine environment and developing areas of marine farming. MAS NMR methodologies for marine algae/seaweed characterization and products extracted hereof have been described (Gaëlle et al. 2015; Salomonsen et al. 2009) and solid-state NMR techniques could be used further to understand the marine nature and potential food usage of the hidden treasury of marine life. A recent example is the detailed interspecie phytoplankton variation in lipid and carbohydrate profiles observed between whole marine micro-algal cells and fresh water species. By using ^{13}C-labelling and a combination of ^{13}C MAS NMR and RINEPT NMR, Arnold et al. (2015) observed differences in cell wall components, storage carbohydrates and membrane lipids.

In the development of an "all-in-one" solid-state probe, the Canadian team of Prof. Simpson (Mobarhan et al, 2016) and Bruker BioSpin performed experiments with live intact fresh water shrimp (*Hyalella Azteca*) grown on a ^{13}C-labelled feed. The results from the Comprehensive Multiphase (CMP) NMR experiments hold promise of a new combined set of MAS NMR experiments where both liquid metabolites (intrinsic dissolved cellular amino acids), semi-solid and restricted diffusion components (shrimp lipids and proteins) and rigid solids (shrimp chitin skeleton) can be monitored concomitantly using the same rotor and one CMP NMR probe.

The nature of MAS NMR enabling the analysis of intact samples and by other methods inaccessible solid materials has opened for the exploitation of biomaterials in bioprocesses like bio-ethanol production. An example is the use of MAS NMR in analysis of the cellulosic, hemi-cellulosic and lignin containing materials (Ragauskas et al. 2014). Furthermore, MAS NMR may be a potential tool for the analysis of side-streams and waste materials from the food industry, and thereby assist in obtaining valuable nutrients or high value biodegradable materials. This is anticipated to be of increasing value in a world where we need to feed a fast-growing population from the earth's resources. Aerobic microbial decomposition of different types of waste has been followed and characterized by MAS NMR (Piterina et al. 2009). Utilization of side-streams from food production has recently been exemplified by Marino et al. (2015), who isolated microcrystalline cellulose from citrus waste biomass

and converted the citrus fibers by use of a microorganism to nano-cellulose, and structural analysis was performed by XRD, FTIR and MAS NMR.

In summary, MAS NMR possess the potential to assist in solving many different food-related challenges, e.g. to differentiate closely related varieties, improving nutritional value, and exploring new raw materials. But of utmost importance, MAS NMR methodologies can help food scientists to obtain an increased understanding of the complexity of intact food systems in terms of structure, interactions and alterations during processing of food from farm to fork.

Author contributions HMJ contributed with literature search and drafted approx. 65% of the text. HCB contributed with literature search and drafted approx. 35% of the text. Both authors revised and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest The authors Henrik Max Jensen and Hanne Christine Bertram declare no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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