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REVIEW

Applications of High-Resolution Solid-State NMR Spectroscopy in Food Science

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The principal applications of high-resolution solid-state NMR spectroscopy, in the field of food science, are reviewed, after a short general introduction, mainly focusing on the potential of these investigations, which are, today, routine tools for resolving technological problems. Selected examples of the applications in the field of food science of high-resolution solid-state NMR spectroscopy both in ^{13}C and in ^{1}H NMR particularly illustrative of the results obtainable are reported in some detail.

KEYWORDS: CPMAS NMR; NMR spectroscopy; food science

INTRODUCTION

The applications of high-resolution solid-state NMR spectroscopy in food science have been reviewed to show the potential of these investigations, which can be considered useful to resolve technological problems. Selected examples of application of high-resolution solid-state NMR both in ^{13}C and in ^{1}H NMR illustrative in the field of food science are reported, and a detailed physicomathematical description of solid-state NMR spectroscopy has been purposely avoided, which can be easily found in the general references. Thus, only a short presentation of the technique is made here as an introductory note.

GENERAL ASPECTS OF THE HIGH-RESOLUTION SOLID-STATE NMR SPECTROSCOPY AND APPLICATIONS IN FOOD SCIENCE

Application of high-resolution NMR spectroscopy to the solid state has been newly developed in the 1970s by the work of Andrews and others (*1*−*4*), and it has been recently reviewed in detail (*5*). The first samples studied by this spectroscopy were solid adamantane and solid benzene by ^{13}C NMR.

After these pioneering studies, solid-state NMR spectroscopy advanced rapidly and was extensively used to study the structure and dynamics of a variety of solid systems ranging from catalysts and glasses to natural and synthetic polymers and proteins. Numerous excellent reviews have appeared in the past few years showing how these tools for the study of solids by solid-state NMR spectroscopy can be applied to solving specific chemical problems (*5, 6*).

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As this technique became a routine spectroscopy and lost progressively the difficulties arising from the complicated experimental technical aspects present in its early stage, the number of papers in the literature of applications of magic angle spinning (MAS) and, particularly, ^{13}C NMR with cross polarization (CPMAS) or ^{1}H (HRMAS), in the literature changed. Reports after the 1980s increased quite slowly up to the 1990 and increased rapidly over 1990−2000, particularly in food science. In recent years, 2000 to today, an important increase of studies occurred including many high-resolution ^{1}H magic angle spinning in solid (HRMAS), which has become more productive than ^{13}C CPMAS NMR.

This review attempts to show the utility of high-resolution solid-state NMR spectroscopy for the study of food components and, in many cases, intact foods. There are several advantages and some disadvantages that will be clearly outlined and summarized in the conclusions.

HIGH-RESOLUTION SOLID-STATE NMR SPECTROSCOPY

The simplest NMR experiments performed with solid-state samples consisted of the observation of spin 1/2 nuclei with low gyromagnetic ratios such as ^{13}C, ^{15}N, and ^{29}Si. It is well-known by NMR spectroscopists and, in general, by chemists that, in the solid state, the interactions between nuclear spins and magnetic fields give rise to very broad NMR signals due to the spin−spin interactions. These interactions play a very important role in the line shape of spectra of spin 1/2 nuclei, and many spectroscopic methods have succeeded in manipulating and sometimes eliminating the effects of these interactions.

As an example, the ^{13}C NMR spectrum of a ^{13}C-labeled, at C1 (10%) glycine in water solution (**Figure 1**), shows two resonances. The low-field (left side) one is a triplet due to the CO *J* coupled with the two protons in C2, and the higher field (right side) one is due to the C2 carbons, which give a much

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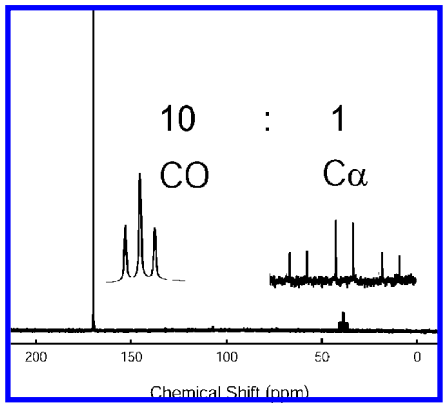

Figure 1. Solution ^{13}C NMR spectrum of C-1, ^{13}C labeled (10%) glycine dissolved in H_2O. Reprinted with permission from ref *5*. Copyright 2002 Wiley.

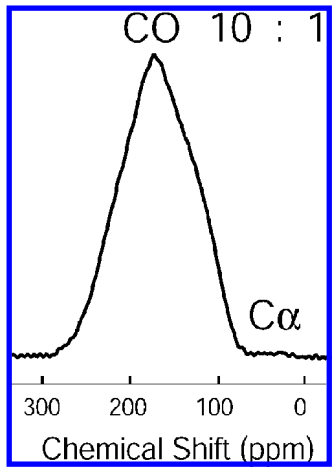

Figure 2. Solid-state ^{13}C NMR spectrum of C-1, ^{13}C labeled (10%) glycine powder. Reprinted with permission from ref *5*. Copyright 2002 Wiley.

less intense quartet shape. The large difference in intensity is due to the fact that CO is ^{13}C labeled, whereas the C2 is in the natural isotopic abundance (1% of the ^{12}C).

The spectrum in **Figure 2** shows the result of the same experiment performed in the solid state. The signals for the carbonyl ^{13}C, very sharp in solution, are now replaced by an extremely broad signal, about 200 ppm, which is difficult to identify as one or more peaks. These broad signals are primarily the result of interactions that are scarcely present and observable only in very particular conditions of orienting media, in liquid-state NMR spectra. The primary interactions in this case were dipolar couplings and the chemical shift anisotropy.

Nuclear Dipolar Coupling and Chemical Shift Anisotropy. The nuclear dipolar coupling arises from interactions between the nuclear magnetic moments of two different nuclear spins. The dipolar coupling depends on gyromagnetic ratios of nuclei, the distance of nuclei, and their orientation by the term of a Hamiltonian $(3\cos^2\theta - 1)$, where θ is the angle between them. Liquid-state NMR spectroscopy, where molecules tumble very quickly, averages to zero these interactions. On the contrary, in the randomly oriented (micro)crystallites present in solid samples, the internuclear vectors are invariant over time. The resonance frequency produced by a nucleus in each crystallite depends on its orientation with respect to the external field.

The introduction of a broadband spin decoupling as in liquid-state NMR removes the heteronuclear J scalar coupling and changes only slightly the spectrum (see **Figure 3**). In the

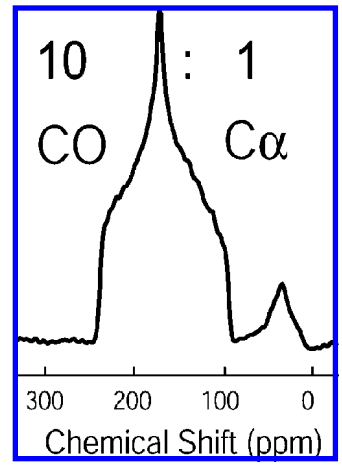

Figure 3. ^{1}H decoupled solid-state ^{13}C NMR spectrum of C-1 ^{13}C labeled (10%) glycine powder. Reprinted with permission from ref *5*. Copyright 2002 Wiley.

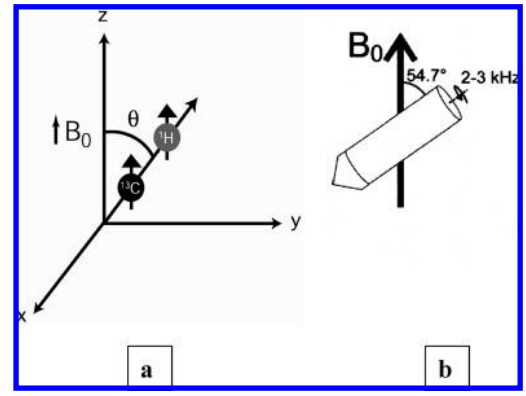

Figure 4. (**a**) θ is the angle between the $^{1}H-^{13}C$ bond vector and the direction of the external magnetic field B_0; (**b**) Scheme of the magic angle spinning. Reprinted with permission from ref *5*. Copyright 2002 Wiley.

spectrum the CO band appears with two large shoulders due to the chemical shift anisotropy. To remove this effect and further narrow the resonance, the fast rotation of sample was introduced.

The rotation at the angle for which the term $(3\cos^2\theta - 1)$ becomes null occurs when θ corresponds to a value of 54.7° (the magic angle). The setting of this angle reduces the dipolar coupling and the broadness of the resonances to its minimum value (see **Figure 4**) with a strong dependence on the rotation speed as shown in **Figure 5** (see caption).

This procedure has a more dramatic effect on the resonances due to the carbon atoms, which are now well resolved in the spectrum, than it does on the signal for the carbonyl carbon atom at about 170 ppm (**Figure 5**, spectrum a).

Chemical Shift and Chemical Shift Anisotropy. At the origin of the chemical shift there is the effect of the magnetic environment in molecules on the specific nucleus. In solution the local magnetic environment around nuclei is determined by the magnetic shielding effects of atoms and electrons of the molecules itself and of the solvent averaged by the several fast tumbling motions. In the solid state the contribution of molecules crystallized nearby in a ordered system has a marked effect on the magnetic shielding and, then, on the chemical shift. The presence or absence of solvent plays as variable contribution. Thus, it can easily occur that a nuclei is particularly sensitive to the state of crystallites, where the molecule is ordered in giving new resonances different from those in solution with a meaningful indication on their crystallinity state and proportional

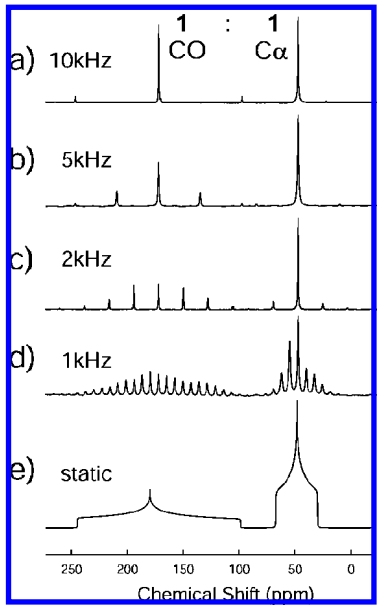

Figure 5. Solid-state ^{13}C NMR spectra of a uniformly ^{13}C labeled (10%) glycine powder sample. The spectra were acquired under ^{1}H decoupling and MAS at the given spinning speeds: (e) static; (d) 1 kHz; (c) 2 kHz; (b) 5 kHz; (a) 10 kHz). As the spinning speed increases, from (e), static, to (a), the highest speed, the envelope of the spinning side bands decreases in number and intensity. At 10 kHz the weak CO spinning sidebands are still present. Reprinted with permission from ref *5*. Copyright 2002 Wiley.

to the amount of the phase in the solid itself or, eventually, giving different resonances in the presence of polymorphic crystallites.

Chemical shift magnetic anisotropy (CSA) arises from small magnetic fields either added to or subtracted from the external field due to circulating currents of electrons. For a non-sp3-hybridized ^{13}C atom, the CSA can be as large as 120−140 ppm.

The rapid spinning, up to tens of kilohertz (this limit is now in further expansion), of a polycrystalline powder sample makes an average of the CSA local fields. The increase of the rotation speed decreases these spinning sidebands and increases more and more the central resonance; this is shown in detail in **Figure 5** (spectra from e, static, to a; the speeds are indicated in the figure and in the caption). At the usual rotation speed values (5−10 kHz) some small sidebands due to the residual CSA (see **Figure 5**, spectrum a) are still present for sp^2 carbons. These residual sidebands can be further minimized by randomly changing the spinning speed during the acquisition, thus averaging out their intensity.

Because magnetic relaxation is usually faster for protons than other nuclei, acquisition times can be quite short but, anyway, must be sufficient for a large recovery of the magnetization along the z axis by longitudinal relaxation. Thus, in high-resolution solid-state NMR spectra, the majority of experiments detects the magnetization transfer to other nuclei such as ^{13}C, ^{31}P, and ^{15}N, resulting in the cross-polarization transfer (CP) from ^{1}H with a spectroscopy with high resolution and an enhanced sensitivity due to the CP. The condition of RF power of the two frequencies at which this transfer occurs is called the Hartmann−Hahn condition ($\gamma_I B_I = \gamma_S B_S$) and produces a flow of magnetization from the highly polarized nuclei (I) to those nuclei with lower polarizations (S) as in the scheme reported in **Figure 6**.

High-Resolution Solid-State NMR Spectroscopy. Recently a large variety of applications of high-resolution solid-state NMR spectroscopy to problems of interest to chemists, physicists, geologists, biologists, engineers, and other scientists has been reported (*6*).

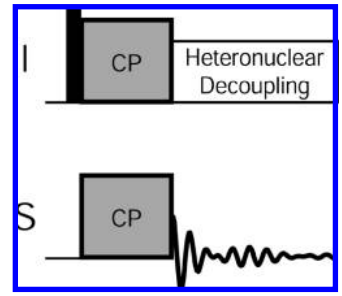

Figure 6. Hartmann−Hahn cross-polarization magnetization transfer. The pulse sequence CP indicates cross-polarization from the abundant nuclei I to the rare nuclei S. Reprinted with permission from ref *5*. Copyright 2002 Wiley.

The focus of the experiments is to reveal the structure of a particular moiety in biological materials, the products of a reaction, the solid-state structures of compounds, the dynamics of molecules in restricted environments, or the connection of microscopic properties to technologically important uses of materials.

This spectroscopic tool gives information readily used by chemists to answer fundamental questions about each of these solid environments. In addition, even though it was first discovered over half a century ago, intellectual challenges in designing and using experiments to separate and/or correlate the components of spectra still occupy the thoughts and efforts of many researchers in spectroscopy. Some of the research lines and their results are here briefly listed.

In the field of synthetic polymers one of the more interesting applications is to characterize the plasticization of polymers. Differences in the mobility of chains are discerned through the experiments. The measure of the magnetic relaxation times to determine mobility is still exploited in the characterization of homopolymers and copolymers and of their blends. Studies of porous materials and catalysis, with particular regard to the study of zeolites, remains an important use of NMR spectroscopy. In both of these fields proton relaxation times and spin diffusion measurements of liquid-crystalline random copolyesters give important information on domain size. For biological solids and biological materials there are a large number of studies to probe ex vivo lipomas and liposarcomas in tissues. The method permitted the detection of a metabolite that could not be detected by the usual nonspinning methods by studying them in the solid state.

CPMAS NMR has a growing number of applications in the study of pharmaceutical solids. Several studies have been reported of ^{13}C CPMAS NMR in the characterization of solid drugs (for a review see ref *7*). Particularly the characterization of polymorphs can be very important in the patent preparation before a drug can be launched on the market.

There are many cases in the literature of oligomers of polysaccharides and particularly cyclodextrins that have been used to transform hydrophobic molecules with interesting pharmaceutical properties, inserting them, by inclusion complex formation, in a polysaccharidic or cyclic moiety suitable to transform it into a soluble formulation.

CP MAS OR MAS NMR SPECTROSCOPY APPLICATIONS IN FOOD SCIENCE

The pioneering works on ^{13}C CPMAS NMR spectroscopy were performed on cellulose, the most important natural

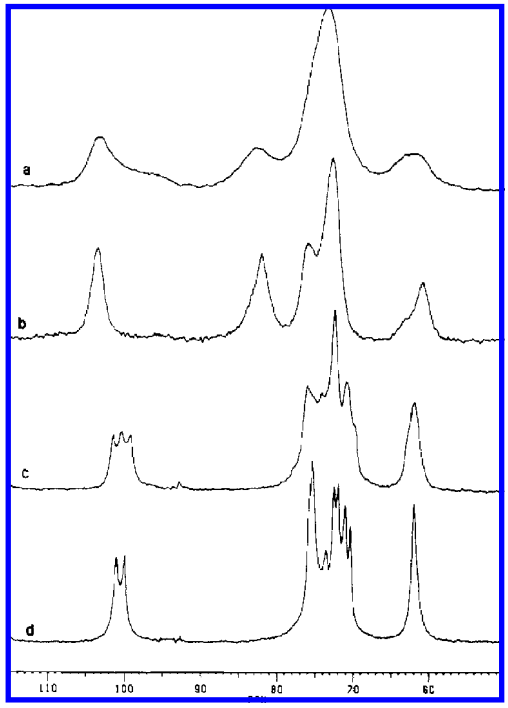

Figure 7. ^{13}C CPMAS NMR spectra of (a) amorphous starch prepared by ethanol precipitaion of gelatinized maize starch, (b) V6 amylose complex with sodium palmitate, (c) crystalline A-type, 1+4 glucan, and (d) crystalline B-type glucan. Reprinted with permission from ref *17*. Copyright 1988 American Chemical Society.

biopolymer, and, in food science, the most important component of fiber(s). Mainly used for the paper industry and extensively studied, primarily for its polymorphism in the solid state, these works were particularly indicative of the potential of this methodology (*8*−*15*), but we will examine in detail only those that are concerned with foods (see below).

On the other hand, the most important biopolymer in food science, either as a food itself or as an additive, is starch, which is, also, a polymer of glucose. Recent studies are here reported in more detail.

CPMAS NMR Spectroscopy of Starch. The nature of starch, its transformations, and its chemicophysical properties either in the granule form or in the form of flour, or, in the separate components (the amylose, the linear 1−4 α-glucose) and amylopectins (1−4 α-glucose with 1−6 α-glucose ramifications) are the research themes of a large number of papers and, among them, in many of these investigations CPMAS NMR spectroscopy or, more recently, ^{1}H HRMAS NMR has been used extensively (*15*−*25*).

CPMAS NMR Spectroscopy of Starch, Amylose and Amylopectins from Different Sources. The starch polymorphism, in particular, has revealed a field in which the CPMAS NMR could give very important contributions to the for characterization of the different allomorphs and, particularly, the resonances of the amorphous state (*17*) (see **Figure 7**, where beautifully well-resolved spectra of the three allomorphs are clearly distinguishable).

The characterization of the behavior of starch depending on the hydration and the retrodegradation process has been considered to be a very important objective for study in the solid state. The more recent developments are herewith reported in some detail.

Retrogradation of Starch. The retrogradation of starch gels was measured using differential scanning calorimetry (*23, 24*), rheology, and NMR spectroscopy techniques. During the initial (<24 h) stage of retrogradation, an increase corresponding to an increase in the number of protons participating in cross-relaxation was observed for all four concentrations studied. During the latter (<24 h) stage of retrogradation, amylopectin recrystallization became the dominant process as measured by an increase for the 25% starch gel, which corresponded to a further increase in mobility. A decrease in the molecular mobility of the liquid component was observed. The value for the solid transverse relaxation time did not change with concentration or time, indicating that the mobility of the solid component does not change during retrogradation. This process of change of solid aggregation is schematically presented in **Figure 8**.

Ball Milling of Starches. Wheat and maize starches, ball milled (*25, 26*), led to damaged starch, with corresponds to losses of short-range crystalline order. The double-helix content was measured by X-ray and ^{13}C CPMAS NMR in the dry starches. The damaged starch in the dry wheat and maize starches was amorphous, with the possibility of restoration by wetting and drying. The swollen gel was quantified as damaged starch using an enzymic assay. A model is presented to explain the observed patterns of damage. It is concluded that amorphous starch within the native granules, which does not include crystalline amylopectin, should be distinguished from amorphous damaged starch because they differ in composition and in their physical properties.

Nutritional Aspects of Starch Polymorphism. A study on the pure components of starch from potato, amylose and amylopectin (*27*) and the mixture of the two, was performed to understand the modifications induced at different levels of the Maillard reaction occurring in the presence of amino acid L-lysine in this study, on the macromolecular structure by ^{13}C CPMAS NMR and digestibility by enzymatic degradation kinetics. The extent of the Maillard reaction has been measured by the furosine method. The reaction with lysine seems to act as a disordering agent; a loss of crystallinity is evident for starch and amylopectin and, in particular, amylose. The polymer digestibility decreases, confirming that structural organization is not the only determinant of starch digestibility.

Along the same line the modification of starch, which is the major component of the polysaccharide fraction of chestnuts, *Castanea sativa* (*28*), has been studied from the point of view of structure by ^{13}C CPMAS NMR and measuring the digestibility to understand the modifications induced by cooking and, specifically, by the Maillard reaction monitored by furosine method. The solid-state phase changes occurring upon chemical modification due to the cooking process were monitored, revealing that large changes are induced in the macromolecular structure of starchy materials and that these changes, also in this case, are correlated with relevant changes of digestibility.

Aging of Starch in Bread. ^{13}C CPMAS NMR was applied to the study of the effects of storage methods and glycerol on the aging of bread crumbs (*29*). The change of shape of C1 peaks from triplet (A-type) to singlet (V-type) was observed. Addition of glycerol changed the carbon peak intensities of fresh and aged breads. Therefore, bread firming in this case was controlled not only by starch retrogradation but also by other events, such as local dehydration of the matrix or gluten network stiffening, corresponding with reconstitution of a double-helical conformation in agreement with DSC results. Factors such as water distribution and migration are the determinants of very important phenomena of aggregation of chains (*29*) similar to those

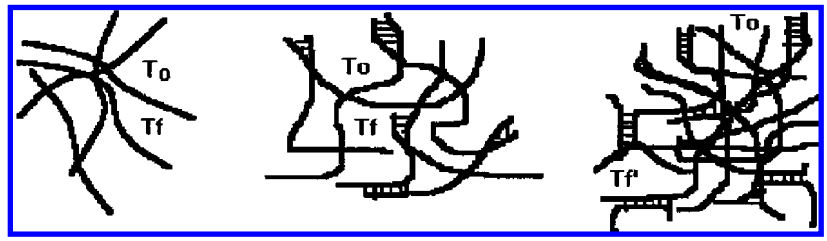

Figure 8. Schematic representation of the network formation between macromolecules and, in this case, the aging of starch gel (adapted from ref *23*).

reported as a scheme for another process in **Figure 8**, also determined by migration of water and formation of a network of close and stiffened polysaccharidic chains.

Polymorphism of Starch. A new improved method for studying native starches (*29*−*33*), considering contributions of the V-type conformation and the amorphous components, separates amorphous and ordered subspectra by evaluating the intensity at 84 ppm. Proportions of amorphous single- and double-helical conformations are determined by the intensity of C1 peak areas. Moreover, the V-type single-helical component increases with amylose content. The method of preparation was found to be more important than the botanical origin in determining ^{13}C NMR spectral features of amorphous samples (*29*).

A series of amorphous samples were prepared from various starchy substrates (*30, 31*), native potato starch, amylopectin, and amylose, the spectra of which have been analyzed by deconvolution of the C1 resonances of the ^{13}C CPMAS spectra. 2D solid-state NMR WISE experiments were performed, and the magnetization curves upon contact time of each C1 component were fitted. Interpretation of the characteristic times derived from fitting have been correlated to the heterogeneity of the samples and to the water molecule distribution in the solid matrix.

Solid-state NMR spectra of amylose (*32*) recorded from highly crystalline ^{13}C-labeled particles allowed the most complete assignment of the ^{13}C CPMAS NMR spectra for B-type starch and amylose.

By using specific sequences some carbon−carbon distances in the B-crystal were estimated in good agreement with the existing model. Measured distances of >0.5 nm required new experiments, which open ways to, especially, assess the carbon−carbon distances in the other A-type polymorph of starch and amylose, for which a slightly different double helix was proposed.

The resonances due to carbon atoms of the amorphous regions of moistened starch granules (*32*) are too broad to be visible in ^{13}C CPMAS NMR spectra. These resonances could be observed in spectra obtained by using a single pulse sequence. This means that the amorphous regions in moistened starch granules are mobile and have properties similar to those of a rubberlike polymer. Relaxation times for resonances assigned to the amylose−lysophospholipid inclusion complexes were shorter than those of the crystalline starch. Relaxation differences were used to generate subspectra of the crystalline starch and the amylose−lysophospholipid inclusion complex.

The results suggest that the inclusion complexes occur in distinct regions of the starch granule.

There are thus three distinct components making up the wheat starch granule: (i) highly crystalline regions formed from double-helical structure starch chains, (ii) solidlike regions formed from lipid inclusion complexes of starch, and (iii) completely amorphous regions associated with the branching regions of the amylopectin component of starch and possibly the lipid-free amylose.

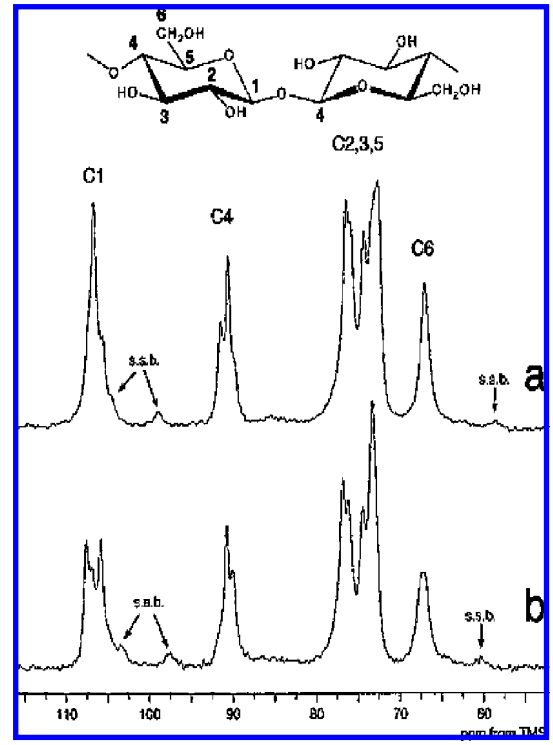

Figure 9. CPMAS ^{13}C NMR spectra of *Cladophora* celluloses at 75 MHz: (**a**) original I α-rich cellulose; (**b**) I β-rich cellulose annealed at 260 °C for 30 min. "s.s.b." indicates spinning sidebands. Reprinted with permission from ref *53*. Copyright 2002 American Chemical Society.

Cellulose and Its Polymorphs from Different Sources. Cellulose is a very important natural polymer with several industrial applications and, moreover, a very important role in nutrition as a food component, usually called fiber(s), together with lignins and similars. The solid state of cellulose is polymorphic and has different crystal forms, which have been studied and characterized (see refs *8*−*15*). The selection of papers herewith reported concerns studies of cellulose(s) extracted from natural alimentary sources (foods) and, in some cases, of the modifications induced by different treatments. In all of the spectra reported as an example in **Figures 9** and **10**, it is noteworthy to observe the narrow (or very narrow) line width of resonances of crystalline or microcrystalline samples.

Soybean cellulose and pectin extracts have been investigated by measuring the kinetics of cross-polarization (*33*). In vitro incubation of cellulose and pectin, from soy hull and endosperm, with sheep fluid rumen was followed. The difference in enzymatic degradation of two pectins with identical monosaccharide composition was explained by the different degrees of esterification. Variable contact time experiments (changing the duration of cross-polarization time in CPMAS NMR) during

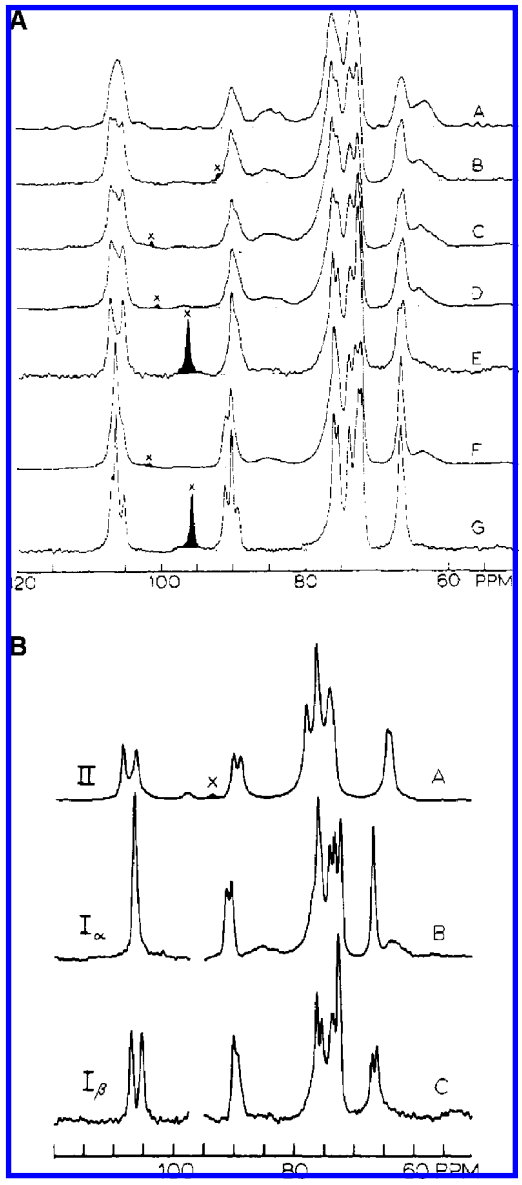

Figure 10. (**A**) ^{13}C CPMAS spectra of various cellulose I materials: A, Norway spruce kraft pulp; B, ramie; C, cotton linters; D, hydrocellulose made from cotton linters; E, low DP regenerated cellulose I; F, *Acetobacter xylinum* cellulose; G, *Valonia ventricosa* cellulose. Note the marked differences in the fine structure of resonances, particularly at C1 and C4, around 105−107 ppm, and around 80 ppm, respectively. Signal to noise varies because some samples were limited in amount. Approximately 2000−5000 scans were recorded. (**B**) Comparison of the ^{13}C CPMAS spectrum of (A) a low DP cellulose II sample and the spectra (B and C) corresponding, respectively, to the two proposed crystalline forms of cellulose I, namely, Iα and Iβ. Spectra B and C were obtained by the low DP *Acetobacter* cellulose. Multiplicities of the C1, C4, and C6 narrower resonances are thought to indicate unit cell inequivalences. Reprinted with permission from ref *13*. Copyright 1984 American Chemical Society.

the incubation revealed that cellulose is degraded in a layer-by-layer way.

CPMAS NMR Spectroscopy of Polysaccharides of Cell Wall, Fibers, and Cuticular Compounds of Different Sources. Cell walls from wheat bran (*34*) were examined to determine their polymer composition. The carbohydrate part of the ^{13}C spectrum was typical of graminaceous cell walls having cellulose and arabinoxylans as their main components. Only a small amount of lignin was observed in the spectrum, but signals from the hydrocarbon chains of cutin were identified.

Strengthening of intercellular adhesion is the phenomenon that compromises the texture and eating quality of potatoes, *Solanum tuberosum* L. (*35*). This effect has been induced reproducibly by exposure to low-pH acetic acid solutions under tissue culture conditions. The resulting parenchyma tissues have been examined by CPMAS NMR to characterize the biopolymer(s) thought to be associated with this syndrome, in particular, the presence of the polyphenol suberin-like aromatic aliphatic polyester. These aromatic domains are composed primarily of guaiacyl and sinapoyl groups (*36*).

Preparations obtained from perennial ryegrass leaves gave "cellulose" residue from untreated ryegrass leaves (*37*). CPMAS ^{13}C NMR spectroscopy was applied to study the structure of the cellulose samples together with X-ray diffraction. It was found that cellulose samples isolated from ryegrass leaves treated and untreated had a much lower percent crystallinity (33.0−38.6%) than that from wood-based fibers (60−70%) and had much shorter fibers (0.35−0.49 mm) than those of either cereal straws, bagasse, or wood. A partial disruption of the hydrogen bonds and microfibrils may be the cause during the dejuicing process by mechanical activity, which results in a decreased cellulose crystallinity and fiber length.

For the study of the accumulation of defensive material grapefruits, *Citrus paradisi*, were injured, inoculated with *Penicillium digitatum*, and incubated under favorable conditions (*38*). Solvent-washed cell wall preparations were further purified using a mixture of cell wall degrading enzymes. The principal chemical moieties of this material were studied by solid-state ^{13}C CP MAS, achieving a complete assignment of the resonances. Cellulose and hemicellulose and, in addition, relevant quantities of cutin were found. The resonances of difference spectrum were attributed to suberin polyester.

Wheat straw degradation (*39*) by *Fibrobacter succinogenes* was monitored by CPMAS NMR to observe the change of the composition and structure of the lignocellulosic fibers using wheat straw ^{13}C enriched during the growth of bacteria on this substrate. A preferential degradation either of amorphous regions of cellulose versus crystalline regions or of cellulose versus hemicelluloses in wheat straw was not observed.

The NMR studies of starches obtained by fruit seeds such as melon and watermelon (*40*) give us a complete description of sample behavior because this spectroscopic investigation allows is to understand the dynamics of molecules in the samples. The results are compared with those obtained by X-ray and thermal analysis.

Pectin samples and the influence of methyl esterification and O-acetylation on chemical shifts of pectin carbons were investigated by ^{13}C CPMAS NMR using model pectate, pectinates, and their acetylated derivatives (*41*). The peak fitting in the region of C-6 resonances gave the values of methylation, amidation, and acetylation degrees.

Suberized cell wall from potatoes, *S. tuberosum* L., were studied by CPMAS NMR (*42*), and the results verified that suberin-like cutin is a polyester containing phenyl-propanoid groups, which are a chemical characteristic of lignin.

The rigidity of individual molecules within a complex structure such as the onion cell wall (*43*), where dry cell walls behave as rigid solids, has been studied. How each polymer contributes to the rigidity of the whole structure was studied by CPMAS NMR. The degree of molecular mobility found was consistent with a crystalline state for cellulose and a glassy state of dry pectins. Hydration produces a small difference in the

rigidity of cellulose, and most of the xyloglucan shared this rigidity, but the pectic fraction became much more mobile. The cellulose xyloglucan microfibrils behaved as solid rods, and the most significant physical distinction within the hydrated cell wall was between the microfibrils and the predominantly pectic matrix. Away from the microfibrils, pectins expanded upon hydration into a nonhomogeneous, but much softer, almost liquid, gel.

Cell wall polymers in growing and nongrowing live celery, *Apium graveolens* L., collenchyma (*44*) were studied by NMR. When the growth of a plant cell ceases, its walls become more rigid and lose the capacity to extend. Decreased polymer mobility in nongrowing cell walls was detected through ^{13}C NMR. Flexible, highly methyl esterified, pectins decreased in relative quantity when growth ceased.

The rigidity and spatial proximity of polymers in sugar beet, *Beta vulgaris*, cell walls (*45*) are consistent with the presence of rigid, crystalline, cellulose microfibrils, mobile pectic galacturonans, and highly mobile arabinans. A CPMAS NMR spectrum showed only the arabinans and very small amounts of hemicellulosic polymers such as xyloglucan, xylan, and mannan and no arabinan or galacturonan fractions.

Xyloglucans with different mobilities present in the primary cell walls of mung beans, *Vigna radiata* L. (*46*), were studied by ^{13}C CPMAS NMR spectroscopy.

Glucose was labeled at either C-1 or C-4 with ^{13}C isotope incorporated into the cell wall polysaccharides of mung bean hypocotyls. Small signals were detected and assigned to xylose of xyloglucans with rigid glucan backbones. The chemical shifts of resonances were assigned to partly rigid xyloglucans surrounded by mobile noncellulosic polysaccharides and indicate that the partly rigid xyloglucans were predominant in the cell walls.

Cell walls of the growing region of cucumber, *Cucumis sativus*, hypocotyls (*47*) were examined by ^{13}C CPMAS NMR. The rigidity within the hydrated cell walls was studied. The xyloglucan microfibrils of cell wall as well as cellulose behave as very rigid solids. Other pectins, including most of the galactan side-chain residues of rhamnogalacturonan, were much more mobile, with a behavior intermediate between those of the solid and liquid states.

The suberized plant cell walls (*48*) have three distinguishing features: (i) a tissue specificity; (ii) a polyaliphatic domain; and (iii) a unique "lignin-like" polyphenolic domain. Potato tubers have been induced to suberize by wounding, and it is an excellent model system. Using specific ^{13}C-labeling studies and specific chemical degradation techniques, the macromolecular assembly process leading to the deposition of this suberized tissue has been studied.

The materials of the pericarp of mature green and red-ripe tomato fruits (*49*) were investigated by ^{13}C CPMAS NMR, revealing compositional differences of various polysaccharidic constituents in the ripening stages. Measurements of the proton rotating frame relaxation times and of the proton transverse relaxation times from CPMAS spectra also revealed changes in mobilities for some pectic polysaccharides upon ripening.

^{13}C CPMAS NMR was applied to obtain spectra of herbaceous plants (*50*) and dietary fiber powders from aronia berry, chokeberry, bilberry, black currant, and apple, and the spectra resulted in the superposition of resonances from different polysaccharides and polyphenolic compounds (*51*). The fiber powders obtained from berries contain significant amounts of anthocyanins, as indicated by their dark violet color, but not verified by chemical shift variations. The anthocyanin-rich extract from aronia berries and its major components, cyanidin-3-*O*-galactoside and epicatechin, have been also studied.

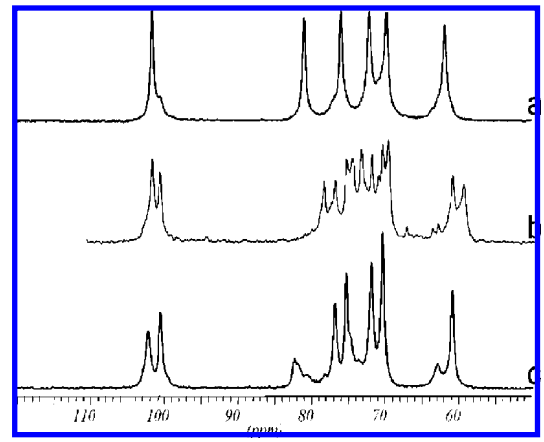

Figure 11. ^{13}C CPMAS NMR spectra of (a) ivory nut mannan recrystallized in the mannan I allomorph, (b) ivory nut mannan recrystallized in the mannan II allomorph and recorded under wet conditions, and (c) cell wall of stems of *A. crenulata* after extraction with 20% NaOH, recorded under wet conditions. Shoulders in some of the resonance peaks are attributed to some minor contaminants or mixed phases. Reprinted with permission from ref *55*. Copyright 2005 American Chemical Society.

The cellulosic fibers of the spines decorating the cladodes of the cactus, *Opuntia ficus-indica* (*50*), was investigated by solid-state ^{13}C NMR together with optical microscopy, scanning and transmission electron microscopy, and wide-angle X-ray. The ^{13}C CPMAS NMR relaxation data showed a strong interaction of the arabinan and the cellulose crystalline domains. Taken together, these observations suggest that the bulk of the spine fibers consists of an intimate composite of cellulose microfibrils embedded in a complex structure.

The resonances of the ^{13}C CPMAS NMR spectra of cellulose triacetates I and II were completely assigned by ^{13}C-enriched allomorphs. Comparison of the spectra of the normal polymer prepared from ramie revealed that all carbons of I appeared as a singlet, whereas those of II, except for C1, are equal-intensity doublets, suggesting that II is made by two nonequivalent glucopyranose residues in the unit cell (*53, 54*) (see **Figures 9** and **10**).

Mannan (*55*) samples having different characters (I, II, and III) were prepared by either (I) desincrusting stems of *Acetabularia crenulata*, (II) acetylating these stems followed by dissolution and recrystallization under deacetylation conditions, or (III) recrystallizing at low temperature the alkali-soluble fraction of ivory nut mannan. ^{13}C CPMAS NMR spectroscopy of mannans revealed that it is a mixture of mannans I and II and that the recrystallized samples were all of the hydrated mannan II family and occurred in a ribbonlike morphology (see **Figure 11**).

In an aqueous environment the samples revealed an additional well-defined perhydrated phase that showed the same ^{13}C solid-state NMR spectrum. Totally dried, their NMR spectra lost their resolution, thus indicating the role played by water for the structural organization of the crystalline and amorphous components of mannan II. In Mexico and Guatemala from prehispanic times the alkaline cooking of corn in a solution of $Ca(OH)_2$ has been used to produce corn-based foods to make corn proteins (*56*) able to remove the corn hull. The main effect of this treatment on the hull is the removal of hemicelluloses and lignin, increasing the hull permeability and the entry of the alkaline solution into the corn kernel. The cellulose fiber network remains crystalline as native cellulose. The alkaline treatment does not

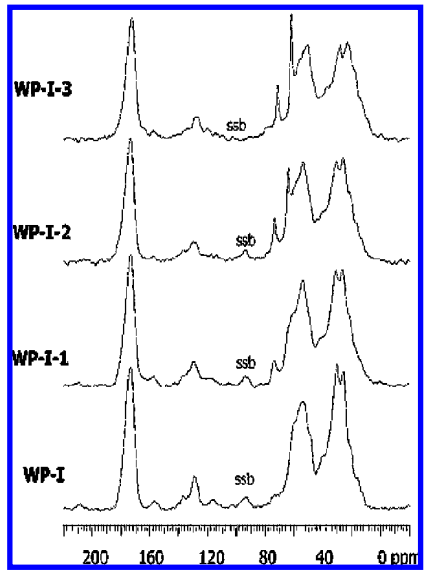

Figure 12. ^{13}C CPMAS NMR spectra of wheat proteins WP-I and WP-I-1, -2, and -3. ssb, spinning sidebands. Reprinted with permission from ref *59*. Copyright 2005 American Chemical Society.

allow the cellulose fibers to swell. On alkaline cooking the hull hemicellulose fraction dissolves, losing its ability to bind calcium and, thus, losing the possibility to incorporate this element into foods.

PROTEINS, THE WHEAT PROTEINS, THEIR HYDRATION AND PLASTICIZATION

A very extensive review of food proteins that reports the results of many techniques including those obtained by high-resolution solid-state NMR has been published (*57*). The effect of hydration on the molecular dynamics of soft wheat gluten (*58*) was investigated on samples of dry and H_2O- and D_2O-hydrated gluten. Measurements of carbon proton CP contact times and relaxation times gave information about mobility. Domains with different structural and dynamic behaviors were identified, as were the different changes induced by hydration. The results are consistent with the "loop and train" model proposed for hydrated gluten.

Wheat proteins processed with glycerol and water as plasticizers (*59*) were studied by solid-state high-resolution NMR spectroscopy, and the structures at molecular level were correlated with the mechanical properties of the materials due to the strong hydrogen-bonding intermolecular interactions between components. High protein content systems show high tendency to be relatively easily plasticized depending also on the high lipid content, but the existence of residual starch would require more plasticizers to reach a similar level of chain mobility (see **Figure 12**).

Investigations on wheat glutenin subunits (*60*) by CPMAS NMR revealed that the hydration induces plasticization depending on disulfide bonds. The study was carried out using dealkylated and alkylated forms of the protein. CPMAS NMR spectra of the samples in the dry state and at two hydration levels (40 and 65% D_2O) were used. ^{1}H MAS experiments were also used on the more plasticized segments (loops), and the results obtained suggest that hydration leads to the formation of a network held by a cooperative action of hydrogen-bonded glutamines. To better elucidate the role of disulfide bonds (*61*), a recombinant 58 kDa peptide corresponding to the central repetitive domain and two synthetic peptides (with 6 and 21 amino acid residues) based on the consensus repeat motifs of the central domain were studied. Experiments in the function of hydration gave information about the role of the protein or of the peptide fractions in plasticization.

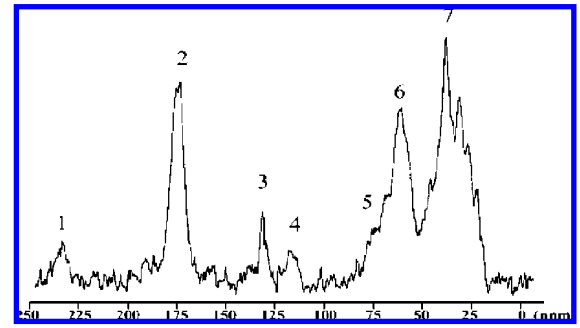

Figure 13. Example of ^{13}C CPMAS spectrum obtained on a frozen muscle sample taken 45 min post-mortem. The main resonances observed are assigned as follows: (1) 235 ppm and (4) 115 ppm, spinning sidebands; (2) 175 ppm, carbonyl carbons; (3) 130 ppm, unsaturated carbons of fatty acids; (5) 72 ppm, C3, C4, C5, and C6 in glycogen; (6) 50−58 ppm, methyl groups of choline and phosphatidylcholine; (7) 30−35 ppm, various saturated carbons of methylene chains of the fatty acids. Reprinted with permission from ref *62*. Copyright 2004 American Chemical Society.

CPMAS NMR Spectroscopy of Freshly Excised Meat Samples. Changes in post-mortem muscle were monitored by ^{13}C CPMAS NMR on rapidly frozen *M. longissimus* muscle biopsies (*62*) together with the measurement of transverse relaxation time. By measuring resonances of glycogen and lactate, the biochemical transformations have been monitored, indicating the metabolic changes in glycogen/lactate ratio. Results indicated a relationship between membrane properties and the amount of water being expelled from muscle cells post-mortem (see **Figure 13**).

^{1}H high-resolution NMR spectroscopy was performed on intact excised rat untreated muscles (*63*). In the high-frequency region of the ^{1}H NMR spectra, resonances have been observed, likely histidine, disappearing in deuterium oxide as solvent, whereas the 6.7 ppm resonance has been attributed to the amino protons of creatine.

CPMAS NMR SPECTROSCOPY OF WHOLE FOODS

The solid-state ^{13}C CPMAS NMR technique has the potential of monitoring the chemical composition and the physicochemical characterization at the solid state of an intact food sample (*64*). This property has been utilized to study the composition of mushrooms of different species, *Pleurotus ostreatus*,*Pleurotus eryngii*, *Pleurotus pulmunarius*, and *Lentinula edodes*, characterized by chemical analysis for protein and dietary fiber components. Solid-state ^{13}C CPMAS NMR spectroscopy reveals a large difference in the ratio between the glucidic and the proteic resonances depending on the mushroom species. An accurate inspection by model compounds and suitable mixtures of proteins and saccharides gives a methodology to interpret these experimental data. A good correlation ($R^2 = 0.93$, $R^2 = 0.81$) has been obtained by comparing the NMR data with the results of the chemical analysis. The results suggest the possibility of performing a taxonomic study and/or a nutritional study on the basis of the ratio between protein and polysaccharide levels determined by NMR. Other studies have been performed on intact seeds in conjunction with magnetic resonance microimaging (*65*). The study was also performed

in the solid state on intact seeds irradiated with γ-rays for improving conservation (*66*) and also to monitor the state of the package and the release of molecules in food (*67*).

Traceability of Foods: the Geographical Source. Durum wheat flours from geographical areas of southern Italy (*68*) were studied by ^{1}H HRMAS NMR. The one-dimensional spectra displayed about 80 resonances, and some preliminary spectral assignment was carried out. A multivariate statistical analysis of spectral data was performed in a trial of discriminating regionality between the samples.

An interesting recent application is constituted by the HRMAS NMR of Parmesan cheese (*69, 70*), where the composition and the chemiometrics of the cheese have been used to determine the geographical origin. A statistical analysis was used to correlate origin with composition.

Other Solid Foods, Polymers, and Sweeteners. Highly crystalline, partially crystalline, and amorphous poly-L-lactide (*71*) were investigated by solid-state ^{13}C CPMAS NMR spectroscopy. The amorphous domains gave broad resonances with Gaussian line shapes, whereas the crystalline domains have narrower resonances with a high degree of Lorentzian character. The spectra of highly crystalline poly-L-lactide have distinct resonances indicating five or more crystallographically inequivalent sites.

Neotame is a common peptidic sweetener (*72*) and, then, can be considered as food, even if its more important utilization is in the pharmaceutical industry. In this last field polymorphism in the solid state is a very common phenomenon, but it is particularly important in correlation with its pharmaceutical biovailability. In fact, the solid form can dramatically affect the physical and chemical stability of the drug. Three of the multiple crystalline and amorphous forms of the artificial sweetener neotame have been described to determine the amount of each polymorphic form with the amount of amorphous forms.

It is also well-known in the pharmaceutical industry that in some cases milling, pressure, and other mechanical factors may deeply influence the solid state and induce polymorphism (*73*). In the field of foods only one case has been reported for trehalose, the well-known bacterial sugar that acts as cryoprotectant. In this case the quantification of the amorphous form in the solid state has been made.

The role of moisture content or water activity of the glucose−water system based on plasticization by moisture (*74*) needs a direct linking between the flexibility of a matrix and the mobility and reactivity of small solute molecules in foods. A CPMAS NMR was applied to study glucose mobility in the solid state over a range of water activities and in matrices with different glass transition temperatures. Results showed that, in a caseinate matrix, compared to a control, addition of glycerol yielded the highest glucose mobility and lowest glass transition temperature (T_g), wheereas addition of sorbitol also increased mobility and lowered T_g. Consequently, plasticization by either moisture or these humectants increases the mobility of small solute molecules such as glucose.

PRACTICAL PROTOCOLS FOR SOLID-STATE NMR SPECTROSCOPY

The practical aspects of this spectroscopy are presented in detail in chemical journals (*75, 76*). The technical requirements are, now, not very complicated. Recent advances by large factories include the development of new probes and rotor-controlling units that are much more reliable and programmable. Thus, the procedure has become much easier to use and friendlier than in the past.

In conclusion, there are a large variety of research themes for which high-resolution solid-state spectroscopy has given valuable results for the understanding of chemical, physical, and biological phenomena occurring in foods. The use of this methodology allowing the study of also intact samples or whole food samples is diffusing rapidly in the world. There are many research centers and facilities where food science and the advancement of this technique are in strict contact for the development of routine uses in this field.

There are some points that must be outlined:

(i) The method is nondestructive, leaving the sample intact for other analyses, and it is important to note that no derivatization is required to perform experiments.

(ii) It has a very high potential to discriminate between crystallinity, its domains, and the polymorphic behavior in samples. The limit is that a very good spectral resolution is required for these studies, which is achievable mainly from the presence in the sample of naturally occurring high-crystallinity domain(s). The low-crystallinity (or amorphous) regions give broad, or hugely broad, spectra, which are scarcely useful for characterizing one or some particular phase in the solid. Some further progress may come from ^{1}H HRMAS in the future, which has high potential to achieve a better resolution but requires some instrumental improvement (the rotation speed is much higher than usual).

(iii) The components of foods that have the higher tendency to be crystalline or microcrystalline or to have crystallinity in their polymorphic forms or with forms close to a high crystallinity are the saccharides and, more significantly, hydrated polysaccharides. In foods these components are mainly starch and cellulose. Their simple spectra and their sharp or very sharp resonances shown in some examples above indicate that they are food components, which gives more reliable results in the study by CPMAS NMR.

(iv) In this field of NMR spectroscopy, crystallinity of intact sample is a very critical factor, much more than the magnetic field strength of solution NMR. In fact, in solids, chemical shifts of resonances are mainly due to the magnetic environments created by nearby molecules in the molecular order of crystallites randomly oriented in a multicrystalline sample. In fact, the orientations of crystallites in the sample has a statistical distribution, giving in the absence of MAS rotation a Gaussian distribution of lineshapes. This means that the increase of the field increases the chemical shift dispersion and, then, that the resonances of a poor crystalline sample, or, in the worst case, of amorphous samples, give broad or very broad resonances, becoming sometimes so large as to be beyond detection.

(v) Foods containing proteins and fats are very difficult to crystallize. In general, these samples are known to crystallize with high difficulty and, in the solid state, their spectra have been, until now, poorly resolved without isotopic labeling and, then, poorly indicative. The newly developed ^{1}H HRMAS has already given very important results. Many others will be reported in the future due to advances in high-speed rotors and better electronics.

(vi) The results reported indicate that the field in which the best results are achievable is that of saccharides and polysaccharides, both as matrix and as additive. Further progress in development with the more recent HRMAS technique will extend its capability in food science to obtain the best results in many cases.

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