

Quality control of agricultural products using magnetic resonance sensors¹

Controle de qualidade de produtos agrícolas através de sensores de Ressonância Magnética

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HIGHLIGHTS:

Magnetic resonance sensors are powerful for quality control of agricultural products.

Maize seeds are examined using magnetic resonance imaging and relaxometry spectra.

A review of magnetic resonance applications in agricultural products is presented.

ABSTRACT: Nuclear magnetic resonance (NMR) is a spectroscopy technique widely used by chemists and physicists to determine the chemical structure of molecules that was adapted to generate imaging, known as nuclear magnetic resonance imaging (MRI), which is widely used in medical diagnosis. The importance of NMR in chemistry, physics, medicine, materials, and agriculture has been recognized with several Nobel Prizes in Physics, 1952, Chemistry, 1991 and 2002, and Medicine in 2003. Therefore, NMR can be applied to obtain: i) imaging of the human body, animal and materials; ii) high-resolution spectra to obtain structural and dynamical information of chemicals, materials etc.; and iii) quantitative and qualitative information of chemical composition of products such as food and agricultural products, using low-resolution relaxometry. High-resolution NMR and MRI have been applied in agri-food products, mostly as a research tool as they typically rely on expensive and bulk instruments, which restrict their uses in routine applications. The NMR sensors that have been more frequently used in agri-food products are based on low-resolution or low-field or time-domain NMR (TD-NMR) instruments. These low-cost instruments have been used for qualitative and quantitative analysis of agri-food products such as intact seeds and grains, intact fruits, meat, oils, and processed foods. In this paper, an overview of the NMR techniques and its main instrumentation aspects are presented, and some applications of TD-NMR and MRI in the non-invasive analysis of food, seeds, and others agricultural products are discussed.

Key words: nuclear magnetic resonance, time domain, seed analysis

RESUMO: A ressonância magnética nuclear (RMN) é uma técnica de espectroscopia amplamente utilizada pelos químicos e físicos para determinar a estrutura química de moléculas que foi adaptada para gerar imagens, conhecidas como tomografia por imagem de ressonância magnética nuclear (IRM), amplamente utilizada em diagnóstico médico. A importância da RMN na química, física, medicina, materiais e agricultura tem sido reconhecida por vários Prêmios Nobel de Física, 1952, Química, 1991 e 2002, e Medicina em 2003. Portanto, a RMN pode ser aplicada para obter: i) imagens do corpo humano, animais e materiais; ii) espectros de alta resolução para obtenção de informações estruturais e dinâmicas de produtos químicos, materiais etc.; e iii) obter informações quantitativas e qualitativas da composição química de alimentos, produtos agrícolas, utilizando relaxometria de baixa resolução. A IRM e a RMN de alta resolução tem sido aplicadas em produtos agroalimentares, principalmente na pesquisa, uma vez que normalmente dependem de instrumentos caros de grandes dimensões, o que restringe a sua utilização em aplicações rotineiras. Sensores de RMN que têm sido utilizados com mais frequência em produtos agroalimentares baseiam-se em instrumentos de baixa resolução ou de baixo campo ou domínio de tempo TD-NMR. Esses instrumentos de baixo custo tem sido utilizados para análises qualitativas e quantitativas de produtos agroalimentares, como sementes e grãos intactos, frutas intactas, carnes, óleos e alimentos processados. Neste artigo, é apresentada uma revisão das técnicas de RMN, seus principais aspectos da instrumentação, e discutidas algumas aplicações de TD-RMN e IRM na análise não invasiva de alimentos, sementes e outros produtos agrícolas.

Palavras-chave: ressonância magnética nuclear, domínio do tempo, análise de sementes

INTRODUCTION

The most well-known application of Magnetic Resonance (MR) technology to the general public is in medical diagnosis imaging. There is a variety of MR sensors which have been projected for different applications, such as: i) to generate internal image; ii) to acquire high-resolution molecular chemical information; and iii) to acquire low-resolution relaxometry spectrum. While high-resolution and magnetic resonance imaging (MRI) have found applications in food and seed analysis, they typically rely on costly and large instruments, which restrict their practical uses in industrial application of agricultural products.

In recent years, there has been a proliferation of novel low-cost instruments and sensors utilizing MR technology to evaluate general biomaterials, such as seeds, plants, meat, polymers, oils, and processed foods, for industrial process control and quality analysis with medium-resolution spectra (Hills, 2006; Jacobsen, 2007; Blümich et al., 2014; Mitchell et al., 2014; Zaleskiy et al., 2014; Lysak et al., 2023). The quality and the production cost of these instruments have significantly decreased, removing the old barriers of being a high-cost, complex and oversized technology (Moraes & Colnago, 2022).

In this paper, an overview of the nuclear magnetic resonance (NMR) techniques and its main instrumentation aspects are presented, and some applications of TD-NMR and MRI in the non-invasive analysis of food, seeds, and general agricultural products are discussed.

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

The NMR phenomenon has been used by chemists and physicists as a spectroscopy technique to study the structure and dynamics of molecules and their interactions since the 1950's, and in the 1970's it started to be used as an imaging technique, known as magnetic resonance imaging (MRI), which is widely used in medical diagnosis.

MRI is the most known application of the NMR phenomenon to the general public (Hills, 2006; Jacobsen, 2007; Blümich et al., 2014; Mitchell et al., 2014; Zaleskiy et al., 2014). The high-resolution images of MRI or also called MR tomography allow detailed analysis of anatomical structure of human body and even monitoring of biochemical processes of different organs. These images are obtained in a non-invasive way, without using ionizing radiation, unlike the radiation used in X-ray imaging (Gil & Geraldes, 1987). The discovery of MRI was awarded with the Nobel Prize in Medicine in 2003.

NMR applications in chemistry, physics, materials, and agriculture start circa 1950 and were so important for these areas that several scientists were awarded with Nobel Prizes of Physics, 1952, and Chemistry, in 1991 and 2002. Therefore, NMR can be applied to: i) obtain imaging the human body, animal and materials; ii) to acquire high resolution spectra that contain structural and dynamical information of chemicals, materials etc.; and iii) to obtain quantitative and qualitative information of chemical composition of homogeneous and heterogeneous products such as food and agricultural products,

using low-resolution relaxometry.

The word 'nuclear' is omitted from the name of the MRI technique when used in medicine because it could lead patients to believe that it uses ionizing radiation such as X-rays or Gamma ray, used in radiotherapy. In the NMR acronym, the term 'nuclear' refers to the use of a magnetic property of the nuclei of atoms, known as nuclear spin, and has nothing to do with dangerous radiation or radioactivity (Gil & Geraldes, 1987; Hills, 2006). In fact, NMR analyses are performed using radio waves, similar to common radio communication, which are the longest electromagnetic waves, i.e., with the lowest energy and do not harm live organisms. Therefore, NMR is a safe technique and is used in the analysis of all kinds of materials, including humans and food.

There is a variety of NMR equipment, which have been projected for different applications, such as: i) generate internal image; ii) acquire high-resolution chemical spectrum; and iii) acquire low-resolution relaxometry spectrum. The theoretical fundamentals of these instruments are almost the same, based on the observation of the signal of the nuclear spin, with some differences in the instrumentation, and a major distinction in the cost of the instruments and their maintenance.

While high-resolution NMR spectroscopy and MRI have found applications in food and seed analysis, they typically rely on expensive and bulk instruments, which restrict their practical uses in industrial application of agricultural products. In recent years, there has been a proliferation of novel low-cost instruments and sensors utilizing MR technology to evaluate general biomaterials, such as seeds, plants, meat, polymers, oils and processed foods, for industrial process control and quality control. The performance and cost of these instruments have significantly decreased, removing the old barriers of being a high-cost, complex and oversized technology (Zaleskiy et al., 2014; Anders et al., 2021).

Most non-invasive NMR analyses of seeds, food and general agricultural products are performed using low-cost instruments called Time-domain NMR (TD-NMR) or Low-field NMR (LF-NMR) or Low-Resolution NMR (LR-NMR) (Colnago et al., 2021). The advantages of these techniques are that they are based on bench-top low-cost instruments, capable of obtaining low-resolution relaxometry data able to obtain a variety of information about fat and water content in seeds and food products, sugar content in fresh fruits, injury in fruits, meat quality among many other applications.

NUCLEAR MAGNETIC RESONANCE ANALYSIS OF AGRI-FOOD PRODUCTS

NMR became practically useful circa 1950's when it was demonstrated that different NMR signals (spectra) could be obtained, and these signals could be used for determining the chemical structure of organic molecules found in living organisms, pharmaceuticals, agricultural pesticides, petroleum and its derivatives, plastics, and many other natural and synthetic products (Mitchell et al., 2014).

These instruments are known as high-resolution NMR spectrometers (HR-NMR). The application of HR-NMR in these areas reduced the time required to determine the

structure of new chemical substances, thus speeding up the development of new medications and agricultural pesticides; for example, the process of determining organic molecule structures was the slowest step in these developments.

More recently, HR-NMR has been used to study the metabolic profiles (metabolomic analysis) of agricultural products such as animal blood and urine, wines and other alcoholic beverages, fruit juices, meat extracts, and many other agricultural products (Ocampos et al., 2024). The advantage of metabolomic analyses by HR-NMR is that it provides the concentration of all components in a sample in a single rapid analysis (minutes). In this analysis, it is possible simultaneously determine, for instance, in the case of blood: glucose levels, urea, HDL, LDL, etc., and in fruit juice: the content of each sugar (glucose, fructose, sucrose, etc.), organic acids, amino acids, among many other substances (Malheiros et al., 2021; Souza et al., 2023; Spricigo et al., 2023).

Therefore, these HR-NMR analyses are still primarily used in scientific research in agriculture because the instruments are expensive (they can cost up to millions of dollars), require large and sophisticated laboratories, and need specialized operators. Some direct applications of HR-NMR in agriculture began in the early 1960's when it was used to determine the oil content in intact maize seeds (Conway & Earle, 1963). This application facilitated the genetic improvement of oilseeds because the seeds themselves, when analyzed (non-invasively), could be used for breeding programs. Furthermore, the analyses were fast (a few minutes per sample), whereas the chemical methods used until then took several days per sample.

In the 1960's, the development of low-cost time-domain NMR (TD-NMR) devices focused on applications in the food and agriculture industries began. The major advantage of these devices, compared to HR-NMR and MRI, is their low cost, including maintenance, as they do not require liquid

helium and liquid nitrogen for cooling and can be installed in conventional, less complex laboratories.

The TD-NMR instruments have gained diverse applications in research and development as the new instruments incorporate new technology of magnets and digital electronics, which allows the use of sophisticated pulse sequences to measure transverse (T_2) and longitudinal (T_1) relaxation times, self-diffusion coefficient etc., which can provide qualitative and quantitative information about several physical and chemical properties of agri-food products. The advantages of TD-NMR, when compared to traditional physical and chemical methods used in agriculture, include rapid analysis (seconds to minutes), and the ability to analyze intact seeds, fresh fruits, meats, and even foods in commercial packages (Moraes & Colnago, 2022). Moreover, TD-NMR analyses do not use chemicals and, consequently, do not generate toxic waste. Currently, many of these devices come with advanced automation, online analysis and calibration systems, allowing simple and rapid analyses.

Figure 1 illustrates the scheme of a general NMR device (MRI, HR-NMR or TD-NMR) and its operation. The NMR instruments are composed of: the magnet (Figure 1A), which magnetizes the nuclei of the sample to be analyzed; the probe, located inside the magnet, which is an "antenna" capable of transmitting and receiving radio wave signals from the sample; the electronics (Figure 1B), consisting of a radio wave transmitter and receiver, similar to those used in radio communication systems; and the computer (Figure 1C), which controls the device, analyzes the NMR data, and gives the results.

The sample to be analyzed must be placed within the magnetic field of the magnet (Figure 1D) to align nuclear spins with the magnetic field, resulting in net magnetization M . The radio frequency pulses act as a torque that rotates the magnetization from the direction of the magnetic field (z direction) to xy plane

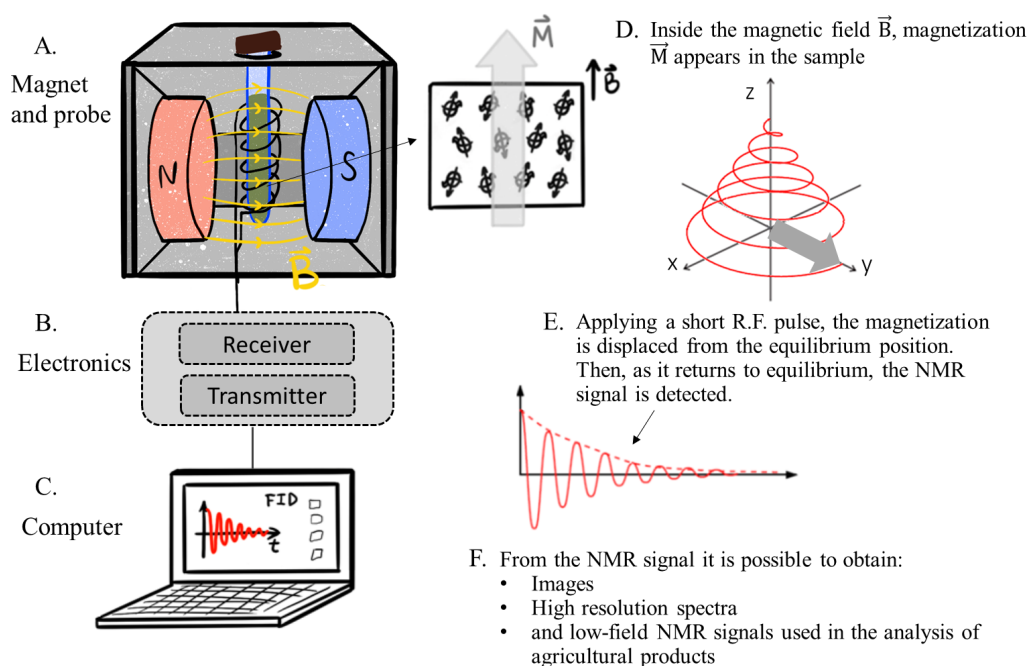


Figure 1. Diagram of a time-domain nuclear magnetic resonance (TD-NMR) equipment and its operation widely used in agriculture to determine oil content, moisture, protein levels, soluble solids, and other characteristics in seeds, food, and general agricultural products

(Figure 1E). Subsequently, the magnetization naturally returns to its original position in the direction of the magnetic field, inducing a signal in the probe, which is the NMR signal known as FID (free induction decay signal). This signal can be used to generate images, high-resolution spectra, or even to measure a quality parameter of agri-food product using low-cost TD-NMR equipment (Figure 1F). Figure 2A shows a photo of one of the TD-NMR devices developed in Brazil, with various applications in agriculture and agro-industries.

Among the applications of TD-NMR in agriculture are the determination of the content and quality of vegetable oils in seeds used in oilseed breeding programs, such as the development of high-oleic peanut cultivars with better physical and chemical properties than regular peanuts, similar to olive oil in terms of qualities (without the flavor and odor of olive oil) (Aramendiz Tatis, 1990; Suassuna et al., 2019; Suassuna et al., 2020; Ribeiro et al., 2021), and analysis of oil content in palm fruits and in several by-products of palm oil industries (Flores et al., 2019). In the analysis of fruit quality, TD-NMR can be used to determine the total soluble solids content ($^{\circ}$ Brix) and whether the fruit is sweet or acidic (Pereira et al., 2013; Moraes & Colnago, 2022). The ratio, which is the ratio between soluble solids and titratable acidity, is a very important parameter for the juice industry as it allows estimating the sensory qualities of the juice, such as its taste (Flores et al., 2016).

In the case of beef, whether fresh or aged, TD-NMR has been used to determine quality parameters such as fat content, tenderness, and moisture. In the case of chicken breast, it has been used to identify physiological anomalies that can affect the acceptability of chicken meat, known anomalies such as striated and woody breasts (Consolo et al., 2020; Consolo et al., 2022; Consolo et al., 2024).

For processed foods, TD-NMR has been used to determine physical and chemical parameters of products directly in commercial package, that is, as they are found on supermarket shelves. In the case of olive oil, the analyses in intact bottle can be used to determine the adulteration with lower-value vegetable oil, a common fraud in Brazil. For mayonnaise and salad dressings, it can determine the fat content and confirm if the packaged product indeed contains the fat content stated on the label (Pereira et al., 2015a; Pereira et al., 2015b). In the case of cream cheese, it can determine the moisture content, fat content, and dry mass, confirming whether it is a traditional or light product (Machado et al., 2022). For jams, it can determine the sugar content and confirm whether it is a traditional or light product as well (Santos & Colnago, 2018). The device shown in

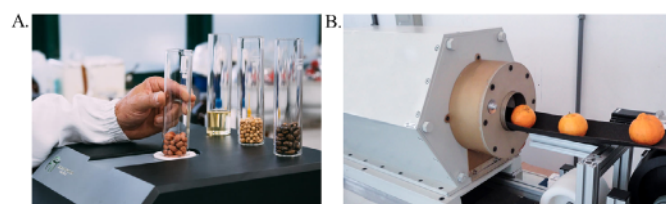


Figure 2. Low-field NMR equipment used in agriculture for the analysis of oil content and quality in seeds (A) and fresh fruits (B), and for the analysis of food products directly in their packaging such as olive oil, wines, milk, tomato sauce, mayonnaise, etc

Figure 2B can also be used to obtain imaging or as an MRI and can be used to obtain internal images of agri-food products. However, the images need a longer measuring time, it is mainly employed in scientific research due to the high cost associated.

In the field of food science, MRI is acknowledged as a noninvasive technique capable of capturing high-resolution images of plant organs and tissue components. Numerous studies utilizing MRI have focused on fruits and seeds (Köckenberger, 2001; Köckenberger et al., 2004; Hernandez-Sanchez et al., 2007; Li et al., 2020; Silva et al., 2021). It is crucial to note that MRI is not a straightforward process; instead, it requires careful optimization of contrast parameters related to the relaxation times T_1 and T_2 , proton density, diffusion, and chemical shifts. Some studies have demonstrated that the application of Fast Low Angle SHot (FLASH) and Zero Echo Time (ZTE) MRI techniques provides favorable contrast for studying seeds with short T_2 signals (Silva et al., 2021).

To exemplify the practical application of MRI, Figures 3A, B and C focus on identifying mechanical, stink bug, and moisture damage in maize seed. In reference (Silva et al., 2021) a similar approach was used in a magnetic field strength of 2 Tesla, whereas in Figure 3, a higher magnetic field of 9.4 T was employed. A Bruker system with Paravision 6.0 from the Weizmann Institute of Science was used, and the Zero Echo Time ZTE sequence was collected with 320 acquisition points, a resolution of 0.1 mm, and a total experiment time of 4.5 hours with a repetition time of 50 ms. The seeds were measured after immersion in water for 24 hours. The cracks and black regions in the images of the seeds in Figure 3 indicate the presence of damage in the seed tissue, which has implications for their physiological quality. This impact can be correlated with specific quality parameters related to vigor and germination potential, typically determined through labor-intensive analyses that may take several days to execute.

Besides the MRI analysis, there is a wide range of NMR instruments available on the market (Mitchell et al., 2014), each type with different characteristics, resolution, and performance. Some of them do not have all the electronics and precise magnets to obtain images of high resolution; however, they are powerful to obtain relaxometric spectra. These simpler spectrometers, of low-field and low-resolution, are much cheaper than MRI systems, being commonly used to analyze agricultural products.

Figure 4 shows an example of these TD-NMR relaxometry signals of maize and peanut seeds. Figure 4A presents the signal

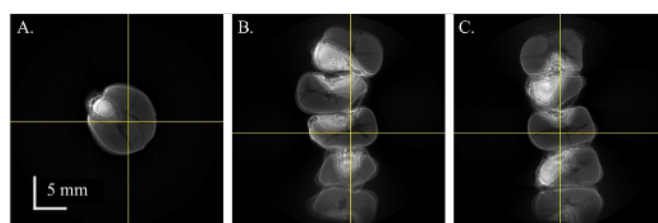


Figure 3. Examples of magnetic resonance imaging of maize seeds, showing the presence of mechanical damage. A, B, and C are coronal, axial, and sagittal views, respectively. The positions of each view are indicated in the other views by yellow lines. These images were acquired using a 9.4T Bruker MRI instrument with Paravision 6.0 software at the Weizmann Institute of Science, Israel

obtained by the pulse sequence Carr-Purcell-Meiboom-Gill (CPMG), and Figure 4B presents its respective Inverse Laplace Transform (ILT) relaxometry spectra. By these spectra, it is possible to identify several internal characteristics of the analyzed samples, and several studies demonstrate the uses of chemometrics, principal components analysis (PCA), partial least square (PLS), and several methods of artificial intelligence (AI) to estimate and determine sample proprieties.

Results presented in Figure 4 were obtained by a TD-NMR spectrometer of 11.3 MHz (0.27 T for ^1H resonance frequency), SLK-200 (SpinLock™, Argentina), using a 30-mm probe at 30 °C, located at Spectroscopy and Electronic Instrumentation

Laboratory of the Department of Biosystems Engineering LEB - ESALQ - USP, Piracicaba, SP, Brazil. The determination of the transversal (T_2) relaxation times was performed using the Carr-Purcell-Meiboom-Gill (CPMG) techniques. The CPMG sequence was executed using 90 and 180 ° pulses of 9.0 and 18.5 μs , respectively, and echo times of 100 μs , with a total of 20,000 echoes, a recycle delay of 5 s and 256 scans. The ILT spectra were obtained by processing the CPMG signal with the Inverse Laplace Transform method (Moraes, 2021; Cardinali, 2024), using 100 points and $\alpha=1$.

Finally, Table 1 presents an overview of examples of recent studies and methods developed by the research group of

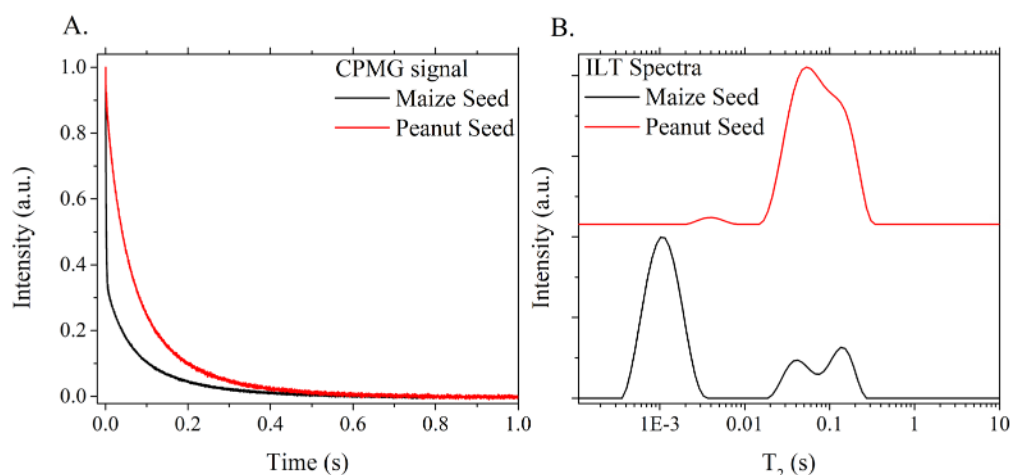


Figure 4. Example of time-domain nuclear magnetic resonance (TD-NMR) signals of maize and peanut seed. A) Carr-Purcell-Meiboom-Gill (CPMG) signals and B) Inverse Laplace Transform (ILT) spectra which make it possible to determine internal properties of the analyzed samples, for example the water environments, oil content and/or presence of tissue damage

Table 1. Some recent Low-Field NMR applications in quality control of agricultural products developed at Embrapa Instrumentação, São Carlos, SP, Brazil

Items / Purpose	MR instrument	References
Seed and grain analysis	Low-Field NMR	Colnago et al. (2007); Colnago et al. (2011); Carosio et al. (2016); Carosio et al. (2018)
Brazil nut oil content	Low-Field NMR	Ribeiro et al. (2021)
Oil content in peach palm	Low-Field NMR	Santos et al. (2023)
Orange juice quality control	Low-Field NMR	Flores et al. (2016); Bizzani et al. (2017); Bizzani et al. (2020a)
Milk and processed cheese	Low-Field NMR	Santos et al. (2016); Nascimento et al. (2017); Machado et al. (2022)
Biodiesel quality control	High and Low-Field NMR	Cabeça et al. (2011); Berman et al. (2015); Meiri et al. (2015); Rocha et al. (2017); Constantino et al. (2019); Cunha et al. (2019); Kock et al. (2019)
Plant and biomass analysis	Low-Field NMR	Wiesman et al. (2018); Bianchini et al. (2020); Aguiar et al. (2020); Colnago et al. (2021); Monaretto et al. (2021)
Plants, seeds, metabolite changes	High-Field NMR	Prestes et al. (2009); Coutinho et al. (2017); Coutinho et al. (2018); Spricigo et al. (2021); Souza et al. (2023); Spricigo et al. (2023)
Fruit quality control, Tomato, banana, plum	Low-Field NMR	Ribeiro et al. (2010); Pereira et al. (2013); Borba et al. (2021)
Mango quality analysis	Low-Field NMR	Bizzani et al. (2020b)
Enzymatic activity in cassava roots	Low-Field NMR	Ferreira et al. (2018)
Jam quality control	Low-Field NMR	Santos et al. (2018)
Chitosan nanoparticles	High and Low-Field NMR	Kock et al. (2016); Facchinatto et al. (2021); Facchinatto et al. (2022)
Beef quality control, aging, fat quantification	Low-Field NMR	Correa et al. (2009); Pereira et al. (2012); Santos et al. (2014); Moreira et al. (2016); Monaretto et al. (2019); Consolo et al. (2021); Consolo et al. (2024)
Myopathies in broilers	Low-Field NMR	Consolo et al. (2020); Consolo et al. (2022)
Quality control of mineral supplements for cattle		Babos et al. (2020)
Olive oil adulteration in full bottles and beverage industry	Low-Field NMR	Santos et al. (2017); Lima et al. (2023)
Non-destructive inline industrial sensor	Low-Field NMR	Andrade et al. (2011); Colnago et al. (2011); Andrade et al. (2012); Colnago et al. (2014)
Noninvasive analysis of intact and packaged foods	Low-Field NMR	Pereira et al. (2015a); Pereira et al. (2015b); Machado et al. (2022); Moraes et al. (2022)

NMR – nuclear magnetic resonance

Embrapa Instrumentação, São Carlos city, SP, Brazil, using Magnetic Resonance technology, with various instruments available in the market, from companies such as Bruker (Minispec mq20™ and Fourier90 spectrometer), Oxford (MQR and X-Pulse), SpinLock (SLK-200), Stellar (FFC NMR), FIT (SpecFIT HR50) and TecMag, besides instrumentation of magnets and coils fully developed by the group.

The application presented covers a large number of agricultural products, with a short description of the item and/or purpose in the first column, the type of Magnetic Resonance instrument in the second column and its respective reference in the third column.

The first three lines of Table 1 present application in seeds, grains and nuts, for determination of oil content and quality. Lines four to six present some application in fruits and vegetables, and its derivatives, such as orange juice, water in plants, quality of tomatoes, internal flesh breakdown in intact palmer mangoes, and enzymatic activity in cassava roots. Lines twelve and thirteen show methodologies developed for quality analysis of meat samples. Finally, other developments are in the analysis of non-destructive, non-contact inline industrial sensor and analysis of intact packaged foods.

CONCLUSIONS

1. The practical application of the discussed methods in industry depends on the total cost associated with each application. Each year, new nuclear magnetic resonance (NMR) instruments are available in the market, with costs constantly decreasing, making many new applications economically viable.

2. Given the potential of the nuclear magnetic resonance (NMR) sensors, the new methods recently developed, the new small and portable magnetic technologies available and the number of companies working on the field, it is believed that these nuclear magnetic resonance sensors will be continuously growing in practical applications for quality control of agricultural products.

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