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Research Article

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Nuclear magnetic resonance rapidly testing method on the moisture content of fresh milk

Ying Liu*, Jiaji Cao, Haowei Zhang, Yajin He and Xinxin Xu

School of Medical Instrument and Food Engineering, University of Shanghai for Science and Technology, Shanghai, China

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ABSTRACT

In this study, with the use of LF-NMR technology, IR pulse sequence and CPMG pulse sequence were applied to detect the changes in the moisture of fresh milk samples, which were stored up at 30°C for 96 hours. Relaxation time signal T_1 and T_2 were measured to analyze synthetically the combination and mobility of water molecules. Furthermore, T_{21},T_{22} proton density were inversed by T_2 to observe the change of bound water and free water, then the total water content can be determined by superposition of inversion signals. Meanwhile, the ratio of T_{21} and T_{22} can be used for quality control and rapid detection of corruption. The experiment results demonstrate that the moisture of milk can be used to determine the quality variation of milk. This research method analyzes and summarizes the change rules of moisture combination state in fresh milk during storage. Consequently, the freshness of milk can be determined rapidly and effectively, and the results will be beneficial to the real-time quality monitoring of fresh milk.

Key words: Nuclear Magnetic Resonance, relaxation time, rapid test

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INTRODUCTION

Fresh milk is the most frequently dairy products eaten in our daily life. Moreover, fresh milk is the raw material of all the dairy products. Therefore, it has a practical significance to detect the freshness of fresh milk rapidly and effectively and to monitor its quality. Fresh milk is nutritious, and is a natural culture medium, which is easy to deteriorate. It is hard to detect the deterioration of the fresh milk unless there is strong smell. Since the moisture content of fresh milk is high (about 87%), and the moisture in food is one of the most important factors that will cause deterioration of the chemical and microbial nature of food, which is directly related to the storage characteristic of food.

It is crucial to find out a rapid detection of the moisture in fresh milk, which can reflect the moisture content and structure accurately to observe its freshness and spoilage. The most of existing methods to determination of water content are time consuming, and vulnerable to be affected by the complicated experimental operation. However, regarding to the Low-Field Nuclear Magnetic Resonance (LF-NMR) technology, there is no limit to the measurement, which can test the sample quickly and non-destructively [1]. It occupies a certain advantage on the detection speed, accuracy and sensitivity, which is an ideal method to measure the moisture content in the food [2].

The LF-NMR technology with a magnetic field strength of 0.5 T is selected in our research. The cost is lower than that of high field NMR. It has been more and more widely applied to the field of food testing due to its advantage of low cost, fast nondestructive detection [3]. CPMG pulse sequence, which can overcome the non-uniformity of magnetic field, was adopted to collect the full and accurate signal T_2, and T_1 values was measured by inversion recovery pulse sequence [4]. Thus the relationship between moisture and other quality characteristics in fresh milk was analyzed comprehensively. The result shows that the moisture content in fresh milk constantly changed along with the extension of the storage time. Moreover, the collected T_2 data was inversed into T_{21}, T_{22} proton density to study the change of bound water and free water respectively, and further observation of the total water content can be determined by superposition of inversion signals. Meanwhile, the ratio of T_{21} and T_{22} can be used to do real-time monitoring of the quality of the fresh milk during transportation or storage and rapid detection of corruption.

EXPERIMENTAL SECTION

The principle of detecting moisture content by LF-NMR

The study of the moisture content of the food is based on the working principle of the Low-Field NMR [5] and uses the activity characteristics of water molecules in magnetic field, thus analyzes the moisture changes of milk. The moisture content can directly affect the average organization's relaxation time, the shorter the relaxation time is, the closer it is combined with the water molecules and macromolecular structure, and if the relaxation time is longer, it means the water molecules is more free. By the comprehensive analysis of T_1 and T_2 binding force and flow properties, we can further observe the existence of free water and bound water [6].

Pulse sequence for milk moisture testing

In the food field, the most common used NMR method is to test T_2 of ^1H proton of the samples, because of its shorter time, a variety of components in the sample can be obtained at the same time. Since not all the needed information can be obtained from T_2 , such as the combination and mobility of water molecules, we can do auxiliary detection to T_1, and make further analysis of the components in the sample and the characteristics of the surrounding environment, thus combining with T_2 and get the information that the sample needs. RF pulse in NMR system is used to generate pulses of radio frequency magnetic field, and thus drive excitation magnetization vector, and produce the power source of the NMR signal. Therefore, the effect of different RF pulse form is different.

In this study, while selecting the pulse sequence for measurement of T_1, we have taken the saturation recovery pulse sequence into consideration, regarding to its small collection of data and poor accuracy of calculation. Therefore, we choose inversion recovery pulse sequence (IR) for T_1 measurement. The CPMG pulse sequence is selected to measure T_2, because the CPMG can overcome the non-uniformity of magnetic field and the effects caused by molecular diffusion. Otherwise, the test object of this study is fresh milk with about 87% of moisture content, and the relaxation is relatively slow. This can avoid the difficulty of CPMG pulse sequence to test fast attenuation material [7], and collect the complete data signal, as well as measure the T_2 value of the sample accurately.

Material and equipment

Purchase a box of fresh milk; Desktop NMR (0.5 T permanent magnet); several test tube; soft dry paper; sucker.

Experimental methods

The experimental object is the fresh milk sample, which is suitable for the detection requirements of LF-NMR technology, because the moisture in the fresh milk is about 87%. Experimental temperature should be selected as 30°C, since the fresh milk will deteriorate faster in 30°C environment, which is easier to experiment monitoring; As well, the LF-NMR instrument can provide 30°C thermotank to facilitate sample preservation, and the magnetic field at 30 °C is more stable, and the collected data will be more accurate. We chose a certain brand of boxes of pure milk to prepare the sample, and sampled 1 ml of fresh milk in test tube respectively for three groups, number them for 1-3 as duplicate samples. In order to prevent water loss produced during experimental operation, we should rapidly take samples by three tubes and place them under 30°C environment after the box of fresh milk was opened. Meanwhile, in order to ensure the comparability of NMR data from the same experimental samples, the same pulse sequence parameters should be used when detecting samples in the same group every 24 hours, and that is to do repeated test 5 times every 24 hours in five different period of each group for samples' T_1, T_2 values (collected by IR and CPMG sequence), take the average value as the each group result of samples in order to reduce the error produced by the experimental process. In the end, through the T_1, T_2 and T_2 inversion data obtained from the experiment, the changes of the free water, bound water, and total moisture can be analyzed in fresh milk during the process of decomposition.

RESULTS AND DISCUSSION

During the process of decomposition for the fresh milk, complex changes have taken place inside the material composition, which is mainly affected by microorganisms. It will cause the constant change of moisture structure and liquidity in fresh milk.

This research adopts the LF-NMR technology for fresh milk moisture determination, and selects inversion recovery pulse sequences and CPMG pulse sequence to test the fresh milk sample T_1 and T_2 parameters and inversion data T_{21},

T_{22} from T_2. Among them, the T_1 and T_2 of hydrogen nuclei can reflect the mobility of water molecules. When the free water content in the organization is high, the average T_1 of the organization is longer; When combined water content in the organization is high, the average T_1 of the organization is shorter; the greater the degree of freedom of the hydrogen protons it is, the greater T_2 is. When water molecules are closely integrated with biological macromolecules such as protein, freedom of the hydrogen proton is abate, T_2 is shorter; when T_{21} representative combines with macromolecular protons closely, it can reflect the change of the combined water; T_{22} represents protons with high degree of freedom, and can reflect the change of the free water.

T_1 changes along with extension of the storage time
Using IR pulse sequences to test T_1 of the 1-3 fresh milk samples, we can get the change process of the longitudinal relaxation parameters T_1 of three groups of fresh milk samples along with the extension of the storage time, shown in Fig.1.

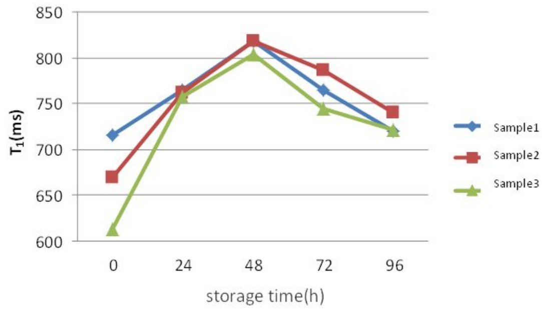

Fig.1: T_1 changes along with extension of the storage time

From Fig. 1, it can be observed that T_1 represents as 'inverted parabolic' shape, which was to say that T_1 firstly increased and then decreased. Comparing with the T_1 experimental data, longitudinal relaxation parameters T_1 increased to about 800 ms after 48h storage of fresh milk, then decreased to around 700 ms. In the original 48h, T_1 increased rapidly, while decreased within 48-96h, and ultimately the T_1 value was greater than the initial value. T_1 can clarify the proton environment from the perspective of molecular, T_1 became longer in 0-48 hours, which means that free water content was more dominant; and T_1 became shorter in 48-96 hours means combined water content was more dominant. At the same time, the amount of T_1 value is inversely proportional to the number of molecules generated by the movement of Larmor frequency, which can estimate that the total moisture in fresh milk firstly decreased and then increased.

T2 changes along with the extension of the storage time
In order to make further study hydrogen protons of different degrees of freedom inside the material, we adopt the CPMG pulse sequence to determinate T2 of the 1-3 fresh milk samples, and get the change tendency of T2 of three groups of fresh milk samples along with the extension of the storage time, shown in Fig. 2.

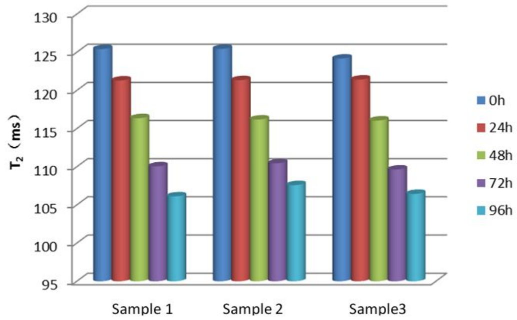

Fig.2: T_2 changes along with the extension of the storage time

Seen from the changing tendency in Fig. 2, we can observe that T_2 of the three groups of fresh milk was gradually diminishing. According to T_2 experimental data, T_2 of three groups of fresh milk samples continuously reduced from around 125 ms to around 106 ms after 96 hours, the decrease of the T_2 values indicated that the degrees of freedom of hydrogen proton was decreased.

Combined with the experimental result of T_1 change in milk, we can make sectional analysis of the change process on the structure of the water. Through the comprehensive analysis of available, when the T_2 values of the fresh milk samples decreased within 0-48 hours, the degrees of freedom of hydrogen protons reduced, which means free water was reduced; while T_1 became longer, it means that the free water content in the water was still greater than the combined water content; When the T_2 values still reduced within 48-96 hours, the freedom degree of hydrogen proton continued to reduce, which means that free water continued to reduce; T_1 became shorter, which means that combined water content was greater than the free water content. However, the total water content increased, which indicated that the combined water was increased.

The different combination state of moisture in fresh milk

In order to observe the change of bound water and free water directly and in detail, we take No.1 sample, import the T_2 data of the fresh milk sample into the T_2-Inverter software and calculate it, we can get map T_{21}, T_{22}, which indicates the density of bound water and free water. As shown in Fig. 3, we can get the inversion signal of free water and bound water obtained from T_2 inversion, and through counting the free water T_{21} and the T_{22} combined water proton density respectively, we can draw the inversion signal trend diagram of No.1 sample. Since the fitting trend of the inversion signal of sample No.2 and No.3 is same as No.1 sample, we can directly select the fitting curve of No.1 sample for analysis.

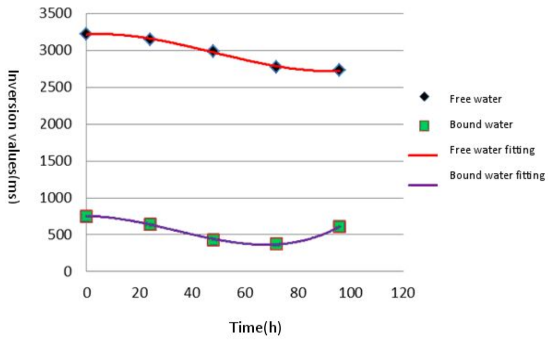

Fig.3: The changing trend of free water and bound water in No.1 sample.

From the analysis, we know that the proton with lower density is bound water T_{21}, proton with higher density is free water T_{22}. Observed from Fig. 3, density of the proton of the combined water T_{21} firstly reduced and then raised, density of proton of free water T_{22} showed a trend of declining constantly. This result further verified the analysis conclusion made from T_1, T_2 test methods.

Get fresh milk and the combined water T_{21} from the fresh milk sample after 96 hours storage time and the proton signal density T_{22} of free water, and analyze the ratio of bound water and free water signal from the fresh milk and metamorphic milk, as shown in Table 1.

Table 1: The ratio of bound water and free water signal from No.1-3 samples

Sample	Time(h)	Bound Water Signal	Free Water Signal	Ratio
No.1	0	645.5	3216.7	0.201
	96	555.2	2725.2	0.204
No.2	0	751.6	3413.9	0.220
	96	615.3	2719.9	0.226
No.3	0	628.1	3308.4	0.190
	96	523.2	2651.2	0.197

Analyzed from the data in Table 1, the ratio of bound water and free water in metamorphic milk is higher than it is in fresh milk. Therefore, combining with the above T_1, T_2 test results, we can get the conclusion that if the bound water in milk increases, the proportion of free water will decrease. At the same time, the ratio of bound water and free water can be used as the standard for quality monitoring and rapid detection of corruption.

CONCLUSION

Compared with the traditional detection method of dairy moisture, Low-Field NMR method has the following advantages: 1) The rapid and non-destructive testing means will not impact on various aspects such as the quality and the flavor of the food during the process of testing. 2) The experiment is easy to operate, and the experiment is of higher repeatability and high precision. 3) There is little change to be affected by the size, appearance, color and luster of the sample, and only a small amount of the sample can be used to determine the food quality. 4) To provide information of proton and molecular level by macroscopic detection can make it more convenient to study the molecular environment of the sample.

Fresh milk is the natural culture medium, which is easy to deteriorate during storage process, and its internal composition changes complicatedly. This research mainly uses Low-Field NMR technology to detect the water change of the fresh milk along with the extension of storage time. The result shows that the total moisture in fresh milk decreased firstly and then increased during the process of metamorphism; among which the free water decreased, and the bound water firstly decreased and then increased. This study is helpful for rapid detection and quality control of fresh milk in the process of storage, and provides a new research idea and effective means for the dairy industry and related regulatory to monitor, control and evaluate the quality of fresh milk, as well as provides a theoretical basis for problems such as further study on watered milk, the shelf life of fresh milk, acceptance check and quality evaluation of fresh milk in the process of transportation.

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