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STUDY OF REGULARITIES OF DISTRIBUTING POWDERED DIETETIC ADDITIVES IN COARSE DISPERSED FOODSTUFFS

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Abstract. An important intervention in the composition of food products is enrichment of food with micronutrients. In this regard, the authors investigated how the additive with the corresponding trace element will be distributed in the food product, and in this case, in minced meat, in order to meet the human needs for microelements.

Micronutrient deficiencies have a significant impact on the nutritional status and health of the population in well developed and developing countries. These deficiencies cause a delay in the growth of children, various diseases, mortality, brain damage, reduced cognitive capacity and the ability of people of all ages. The global scale of micronutrient deficiencies in dietary intakes, in particular the lack of trace elements, has led to the development of powdered dietary supplements containing essential elements that enrich the coarse-type food products to increase their nutritional value. The dietary supplement should provide the daily requirement of trace elements in the human body; therefore, it should be added to the product in a normalized amount and evenly distributed in the product.

Two nuclear magnetic resonance (NMR) and electron paramagnetic resonance (EPR) analysis were performed to determine the distribution of the additive in food products. The analysis was carried out in two stages respectively: study of molecules mobility by measuring the spin-spin relaxation time (T2) and spin-lattice relaxation (T1) on a pulsed NMR spectrometer; establishment of a connection between the exponent of the amplitude of the sample A_0 and its mass. Based on the data obtained, as a result of the measurement, a curve is constructed for the dependence of the amplitude of the echo signal from the value (time interval between the probing pulses). The spin label used in this work is one of the first variants of a paramagnetic probe-an easily accessible transition metal ion Mn^{2+}. According to the constructed graphs and tomograms from the results of the studies, it is possible to study the characteristics of this dietary supplement and the expediency of its use.

Key words. Powdered dietary supplements, nuclear magnetic resonance, electron paramagnetic resonance.

ВИВЧЕННЯ ЗАКОНОМІРНОСТЕЙ РОЗПОДІЛЕННЯ ПОРОШКОПОДІБНИХ ДІЄТИЧНИХ ДОБАВОК У СКЛАДІ ХАРЧОВИХ ПРОДУКТІВ ГРУБОДИСПЕРСНОГО ТИПУ

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Анотація. Серйозним втручанням до складу продуктів харчування вважається збагачення харчових продуктів мікронутрієнтами. У зв'язку з цим досліджено, як добавка з відповідним мікроелементом буде розподілятися у продукті харчування, а в даному випадку у м'ясному фарші, для того, щоб задовольнити потреби людини у мікроелементах. Проведено два аналізи методом ядерного магнітного резонансу та електронного парамагнітного резонансу для визначення розподілу добавки в продуктах харчування. Аналізи проводились відповідно у два етапи: дослідження рухливості молекул шляхом вимірювання часу спін-спінової релаксації (T_2) та спін-градкової релаксації (T_1) на імпульсному спектрометрі ЯМР; встановлення зв'язку між показником амплітуди зразка A_0 і його масою. На основі отриманих в результаті вимірювання даних побудовано криву залежності амплітуди сигналу луни від величини τ_i (інтервал часу між зондувальними імпульсами). Спіновою міткою, яку використано в роботі, є один із перших варіантів парамагнітного зонда – легкодоступний іон перехідного металу Mn^{2+}. За отриманими графіками та томограмами вивчали характеристику даної дієтичної добавки та доцільність її застосування.

Ключові слова. Порошкоподібні дієтичні добавки, ядерний магнітний резонанс, електронний парамагнітний резонанс.

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Introduction. Formulation of the problem

Modern human constantly feels the lack of micronutrients, since during the era of human existence, stereotypes concerning nutrition have significantly changed, and the level of physical activity has decreased. The use of extensive technologies in agriculture resulted in the erosion of soils and the decrease of microelements in them, their low level in drinking water has critically fallen down. Pollution of the environ-

ment by toxicants, active use of mineral fertilizers, pesticides, and herbicides contribute to blocking the absorption of trace elements by the root system of plants. In addition, population of many countries suffers from a shortage of trace elements due to certain geochemical features of the area. Modern technologies of processing raw materials also lead to the loss of a part of trace elements in ready-made food, which became the dominant part of a contemporary consumer basket. Therefore, it is expedient to use dietary supplements, which are the carriers of biologically active forms of irreplaceable micronutrients in products widely used by all groups of the population. An important problem is the study of the distribution patterns of powdered dietary supplements in the composition of poor-dispersion foods.

Analysis of recent research and publications

In economically developed countries, much more attention is paid to changing the structure of food, which is directly related to the health of the nation. Therefore, the problem of ensuring the population with food products is relevant, despite all the achievements of scientific and technological progress [1].

The analysis of the population dietary in Ukraine reveals significant deviations from the formula of rational nutrition - the increased energy value of the diet due to animal fats and carbohydrates, deficiency of proteins, many vitamins and trace elements, as well as dietary fibers. One of the reasons for such imbalance is the food industry's release of products that do not correspond the recommended standards of quality nutrition in terms of nutritional and biological value.

In recent years, there has been a significant upgrade in the range of food products, bringing it closer to the current demands of the population. Increasingly popular are food products enriched with functional ingredients that require the introduction of modern technologies from manufacturers of these products, modernization of technical equipment used for their manufacture, improvement of practical and scientific knowledge of employees involved in the manufacture of food products [2–4].

When organizing the manufacture of food products enriched with physiologically functional ingredients, a number of difficulties arise. It is necessary to choose the correct form of physiologically functional ingredients, carefully control the accuracy of their introduction and uniformity of distributing the dosed components throughout the mass of the finished product to be sure of the components' safety. Ready-made vitamin and vitamin-mineral mixtures, so-called premixes, are developed and manufactured for the direct enrichment of specific foods using similar forms. The use of ready-made premixes significantly facilitates the task of developers and producers of the enriched foods, protecting against possible mistakes and unacceptable combinations in the process of the enriched formulations preparation. The use of premixes, where all the inserted components are thoroughly mixed with each other, ensures their more even distribution throughout the mass of the enriched product than with separate introduction of each component. To ensure uniform distribution of physiologically functional ingredients throughout the product mass, and for the prevention of the ingredients' layering with different bulk weights and particle sizes, the traditional method of multiple gradual dilution of minor components of the formulation by the main components of a dry mixture [5–7] is used.

From the point of view of the safety of physiologically functional ingredients, the most optimal components are dietary supplements containing essential trace elements, as they have evidence base of positive influence on a human body and permission documentation to use for nutrition.

The purpose of the study is to determine uniform distribution of essential trace elements in food products.

The **tasks** of the study are:

1) to obtain layered images of the internal structure of the meat food system;

2) to determine the time of spin-spin and spin-cursor republications using NMR and EPR methods;

3) to determine the role of the amount of water component in the uniform distribution of the powder in coarse disperse systems.

Research Materials and Methods

To determine the distribution of the powdered dietary supplement in foods, two methods of analysis were used: nuclear magnetic resonance (NMR) (Device RE1301, Manufacturer: Ukraine); Electronic Paramagnetic Resonance (EPR) (Device: Tecmag Redstone 1–500 MHz, Manufacturer: Russian Federation). These methods were aimed at obtaining original tomograms carrying information about the molecular mobility of the aqueous medium of a coarse disperse system and the distribution by volume of the metal ion, in which, Mn^{2+} was used as a spin label.

For the investigation, we took mass of the meat stuffing system weighing 100 g and the content of the daily need in K^+ micronutrient, simulated by Mn^{2+} ion, in the human body. For the analysis of NMR and EPR, the mass was divided into 8 contingent parts; each was represented by a parallelepiped. From the obtained parts, three samples were taken and sent for NMR analysis. For electron paramagnetic resonance, predried samples were taken in order to exclude the influence of water on ESR signal.

Nuclear Magnetic Resonance method is widely used to investigate a variety of food products [8]. For this purpose, standard techniques considering all procedures of application and capabilities of the NMR method have been developed.

To evaluate functional and technological properties of the samples, the investigation was conducted in two stages: study of the molecules mobility by measuring the time of spin-spin relaxation (T_2) and spin-grad relaxation (T1) on pulsed NMR spectrometer.

The research of the first stage is based on the sequence of Khan [9]. According to the general theory, magnitude of the signal at the output of NMR spectrometer is proportional to the number of resonating nuclei in the specimen, the angle of deviation of the magnetic induction vector during the alternating field action, and some additional factors. In the selected samples, the nuclei of hydrogen–protons–were resonating nuclei. Investigations of the relaxation time were performed based on the spin echo method.

The following method of determining time (T_2) is used: a sample with the material under investigation is placed in the radio frequency coil, installed in a constant magnetic field. When irradiated with an alternating magnetic field at a frequency of 16 MHz [10], a response arises due to the structure of the system under research.

In the Khan spin-echo method, two pulses with interval τ_i are applied to the sample under study. The first pulse turns magnetic moments to the angle of 90°, and the second – to 180° angle. After the termination of radio frequency pulse within $2\tau_i$ time, an echo signal arises due to the return of the magnetic moments to the initial state under the influence of a constant magnetic field. The formation diagram of the spin echo signal and the type of probe electromagnetic pulses used in the Khan method are shown in fig. 1.

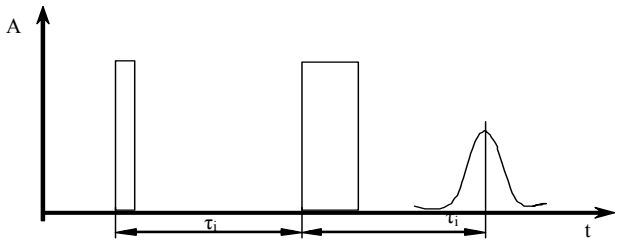

Fig. 1. Formation scheme of a spin echo signal by the Khan method.

It is known that the signal amplitude at the output of NMR spectrometer is determined by the expression:

$$A(2\tau)=A_0 \exp(-\frac{2}{T_2}\cdot\tau), \qquad (1)$$

Where A(2τ) is the signal at the output of the spectrometer, A_0 is initial amplitude of the signal.

To determine the value of the investigated T_2 at the interval between probing pulses equal to $\boldsymbol{\tau}$, A_0 sample, a series of experiments are performed, in which the value of the echo signal amplitude after the influence of two probing pulses with different values is fixed.

Measuring the amplitude of the echo signal at various values of τ_i, the time of spin-spin relaxation was determined and the mobility of water in the sample was evaluated. An example of the oscillograms of NMR signal, on which the value of T_2 is calculated, is shown in fig. 2. The first oscillogram shows the echo signal with a minimal τ_i value. The last oscillogram shows the echo signal at the maximum value of τ_i.

By the formula (1), it is possible to determine the time of spin-spin relaxation and estimate water mobility in the sample. An example of the oscillograms of NMR spectrometer, used for calculating the value of T_2, is shown in fig. 2.

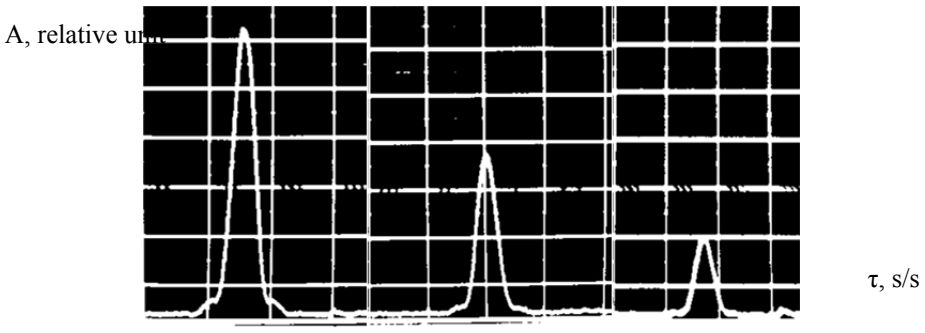

Fig. 2.Oscillograms of NMR spectrometer signals

Based on the data obtained after the measurements, the curve of the dependence of the echo signal amplitude on the magnitude (time interval between probing impulses) is constructed.

An example of typical dependence of the echo signal amplitude on the value of $\boldsymbol{\tau_i}$ is presented in fig. 3.

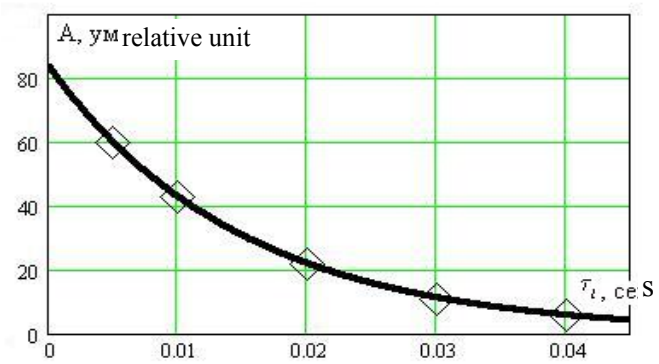

Fig. 3. Dependence of the echo signal amplitude on τ_i magnitude

The dependence A(τ) is exponential. To determine the value of T_2 by the expression (1) based on the curve (Fig. 7d), the standard function *genfit* of the mathematical package MATHCAD was used.

A technique based on the two-pulse Khan method (1.d) was used to determine spin-grad relaxation time (T_1) and has the following form (90°– τ_i – 180°– τ_i – 90°– τ_i –180°–T_0–). The signal amplitude after the action of the first pulse is equal to the of the spin echo amplitude using the Khan method. The spin echo amplitude after the action of the second pair of pulses is determined by the formula

$$A(t_i + 3\tau_1) = A_0(1 - \exp(-t_i + 3\tau_1)/T_1)) \qquad (2)$$

where τ_i is the interval between the probing impulses according to the Khan method, t_i is the interval between the two sequences of the Kang method's probe impulses.

The computer recorded images of the spin echo (in the form of two pulses) at a time equal to τ_i and $\tau = t_i + 3\tau_i$, and displayed on the computer screen. An example of the general type of oscillogram for two different values is shown in fig. 4.

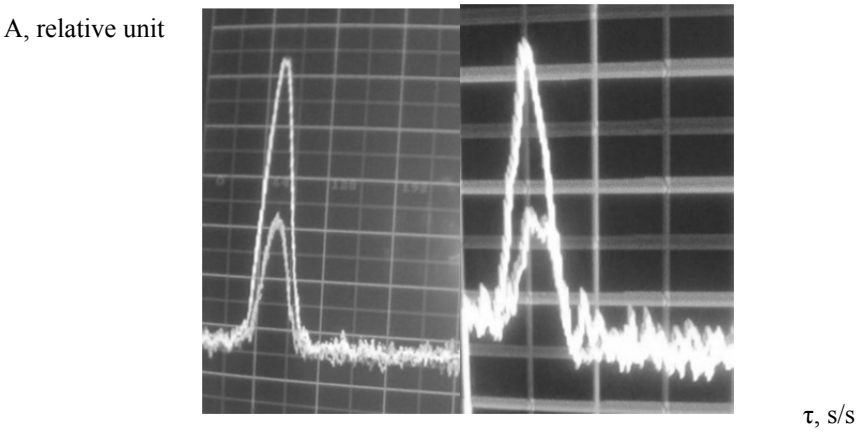

Fig. 4. General view of oscillograms when determining T_1 for two values of t_i

From the above example, it is well seen that the echo signal from the first probing sequence has the same value, and the impulse from the second sequence increases with a change in t_i magnitude.

To determine the value of T_1 by the expression (1) based on the dependence (2), the standard function "genfit" of MATHCAD mathematical package was also used.

One of the molecular-kinetic methods for investigating system water of disperse systems is electron-paramagnetic resonance (EPR) method [11]. Application of ESR spectroscopy in various fields of chemistry, physics, biology and medicine is widely known, and the addition of spin labels, that is, the introduction of paramagnetic probes into the investigated non-paramagnetic system, significantly expanded the possibilities of the method due to unique sensitivity of the spin label to physical conditions of its molecular environment. Thus, this method can be used to study moisture in mono- and poly-molecular sorption.

The spin label used in the work is an easily accessible ion of the transition metal Mn^{2+} [12]. Radio spectrometer RE1301 was used to register the EPR spectra. The spectra were registered as the first derivative of microwave energy absorption by *E* paramagnetic matter under scanning of a constant magnetic field *H*. $MnSO_4$ salt powder, its solution, and meat stuffing system, where aqueous solution of $MnSO_4$ salt was

used as a moisturizing fluid [13–15] and as objects of the research.

The idea of the method is based on the following results and assumptions. Since the absorption spectrum of the microwave energy by paramagnetic is recorded as the first derivative (fig. 5), the first step is to find its original, which can be found by summing the obtained experimental data (fig. 6). The EPR spectrum of $MnSO_4$ salt powder is a broad single line without a superfine structure (fig. 6a). The spectrum of its solution consists of six peaks of equal intensity (fig. 6b). The spectrum from meat nutrition system is a broad single line, which is superimposed on a divided spectrum of six peaks (fig. 6c).

Based on the appearance of the signal of meat nutrition system, it is possible to assume that its spectrum consists of a broad single line without a superfine structure and a separated spectrum of six peaks of equal intensity.

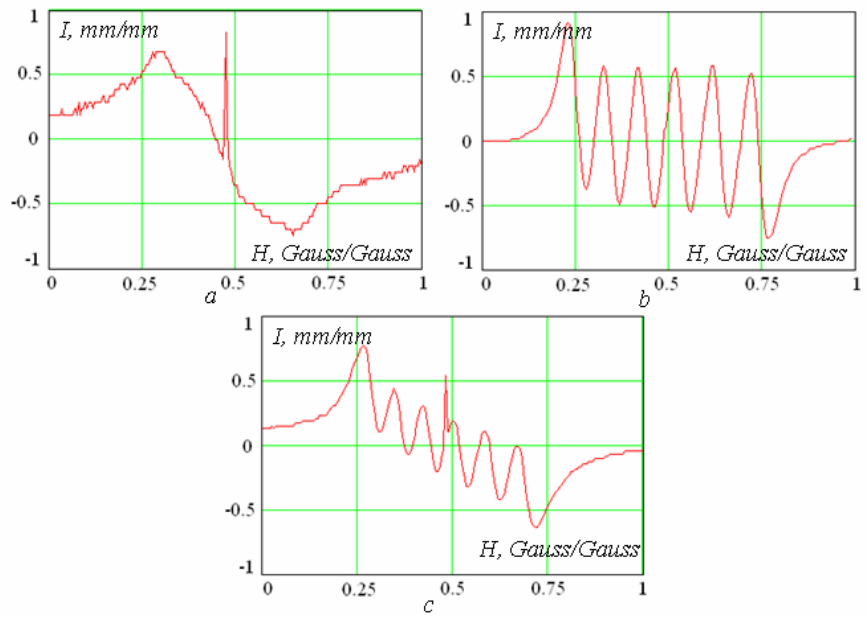

Fig. 5. Spectra for: *a* – $MnSO_4$ salt powder; *b* – its solution; *c* – meat nutrition system

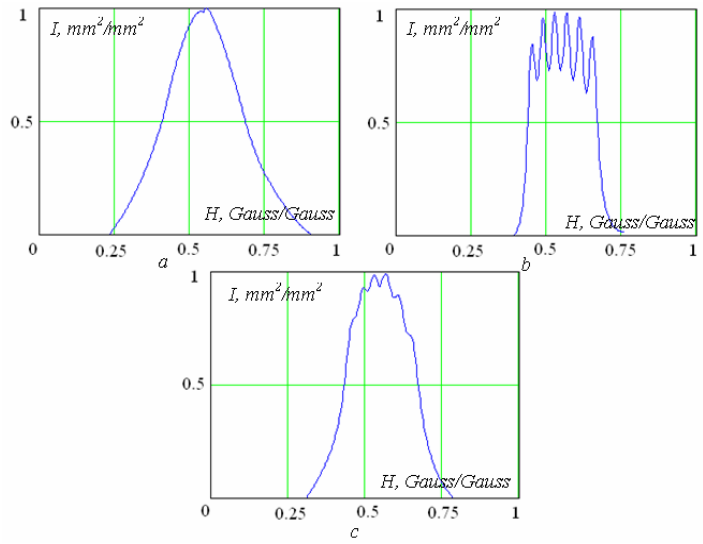

Fig. 6. Integrated experimental data for:
***a* – $MnSO_4$ salt powder; *b* – its solution; *c* – meat nutrition system**

Moreover, the area under the spectrum, i.e., under a wide line and under the spectrum of six peaks, is proportional to the number of resonating spins, and, respectively, the number of Mn^{2+} ions having limited mobility, and Mn^{2+} ions, which are found in the solution. As the water is bound or removed, the solubility of salt decreases, which results in equilibrium shift: to the left with the formation of non-dissociating mole-

cules. This, in its turn, leads to the fact that the area under the single line increases.

$$MnSO_4 \overset{\leftarrow}{\rightarrow} Mn^{2+} + SO_4^{2-}$$

From the theory of EPR method, it is known that the obtained spectra can be described by Gauss (3) and Lorentz (4) equations in the form:

$$I_G = I_0^G \cdot \exp\left(-\frac{(H - H_0)^2}{\Delta H_G^2}\right), \quad (3)$$

$$I_L = I_0^L \cdot \left(1 + \frac{(H - H_0)^2}{\Delta H_L^2}\right)^{-1}, \quad (4)$$

where, I_G , I_L is a current value of the spectrum intensity, relative unit (mm^2/mm^2); I_0^G, I_0^L is a maximum value of the spectrum intensity, relative unit (mm^2/mm^2); H , H_0 is a current value of the magnetic field strength and value of the intensity at which spectrum is maximum, *Gs*; ΔH_G^2, ΔH_L^2 is the line width, *Gs*.

Coming out of it, the next step of the developed method of EPR-spectra analysis is to find basic parameters of the lines under research (spectra of MnSO4 salt powder).

The specified amplitudes are used to find the area under the function consisting of a single line. The area under the spectrum is calculated as an integral of analytic functions in infinite boundaries:

$$S_{cryst} = \int_{-\infty}^{+\infty} f_{1p}(H, I_0^{1p})dH , \quad (5)$$

The area S_{cryst} is proportional to the amount of resonating spins of Mn^{2+}electrons of salt, which is in a crystalline state in the samples under study. The area S_{sol} is proportional to the number of the spins of ion electrons Mn^{2+}in the solution, and, accordingly, the moisture mass that manifests itself as a solvent.

In the work, the area S_{cryst} was calculated for the investigated nutrition systems.

Results of the research and their discussion

As a result of these two studies, original tomograms were obtained at the relaxation time T_1 (fig. 7), T_2 (fig. 8) (NMR) and content of Mn^{2+} (EPR) (fig. 9).

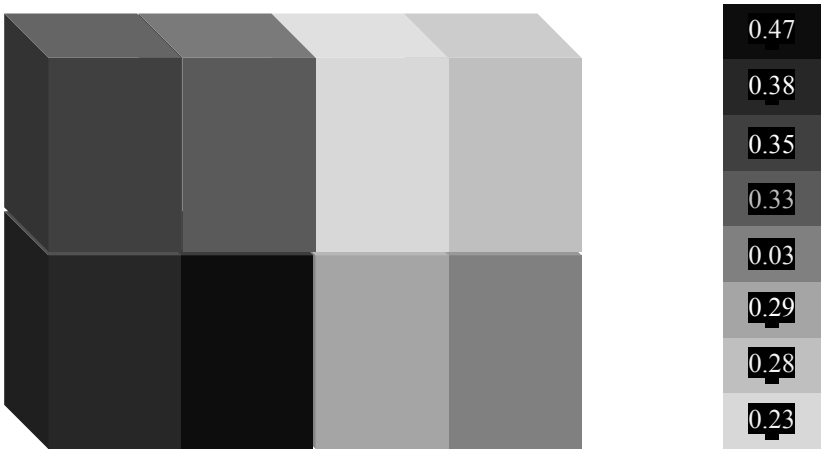

Fig. 7. Time indices of spin-grad relaxation at T_1

Fig. 8. Indices of spin-spin relaxation time at T_2

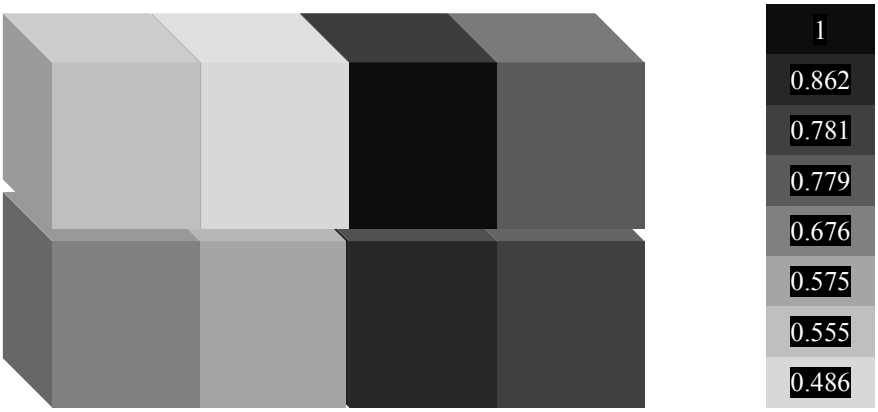

Fig. 9. Indicatorsof S_{cryst} area

Analyzing results of the research of nuclear magnetic resonance, where the time of spin-spin relaxation (T_2) is responsible for the interaction of water molecules among themselves, and the time of spin-grad relaxation (T_1) is the relaxation time with other molecules, in our case, the molecules of powdered dietary supplement, established some difference between these figures. Considering tomograms starting from the upper left sector, it is evident that in the first sample, the interaction of water molecules among themselves is slightly larger, which is 0.085 s, in relation to other sectors in fig. 8, than their interaction with the environment, e.g. Mn, which is 0.35 s and takes the third position according to the amount of spin-grad relaxation time. In the second sample, a greater is the interaction with a dietary supplement and everything that environmental water interacts with, namely 0.33 s, and it is the fourth place by spin-grad relaxation, because the time of spin-spin relaxation 0.081 s takes the third position by the time of spin-spin relaxation. In the third sample, the molecules mostly interact with the molecules taken from the environment, i.e., 0.231 s takes the last place by T_1 indicator. At the same time, there is a significant interaction of water molecules, namely 0.073 s takes the sixth place out of eight in terms of T_2. In the fourth sample, a strong interaction with the molecules from the additive is found, and constitutes 0.285 s. However, in this part of the stuffing system, there is a smaller interaction between water molecules, namely 0.076 s.

The powdered dietary supplement based on Mn is uniformly distributed in the coarse-dispersed meat stuffing system. However, there are differences in some sites, for example, in the fifth sample, which starts with the bottom left sector, in relation to the fourth. A weaker interaction with the environment 0.385 s takes the second place by the value of T_1 indicator, but the maximum is between water molecules, namely 0.062 s. The sixth example differs by smaller interaction between both water molecules and additives 0.471 s – the longest period of spin-grad relaxation, and water molecules among themselves, namely 0.084 s – the second position by the value of T_2. In the seventh sample, the interaction is almost even, the water molecules equally strongly interact among themselves, namely, T_2=0,072 s, and with the environment, T_1=0,292 s. In the eighth sample, an average by its strength interaction is noted between water and additive, namely 0.303 s and less, between water molecules, where T_2=0.085 s is the maximum spin-spin relaxation time.

This happens because the smaller is the time of spin-spin relaxation (T_2), the stronger is the interaction of water molecules with each other, and the smaller is the time of spin-grad relaxation (T_1), the stronger is the interaction of water with the environment: air molecules, powdered dietary supplement, fat, etc.

Due to the fact that a significant number of Ukrainian population suffers from alimentary-dependent diseases, the results are of great scientific and practical value. Products rich in dietary supplements can be produced, both in meat processing enterprises and in restaurants, as well as in food stores, children's, medical and prophylactic institutions.

Conclusions

Consequently, we can conclude that after obtaining a layered image of the internal structure of meat nutrition system, it is found that eight parts, into which a mass was divided, have uneven time of spin-spin and spin-grad relaxation, but this unevenness is small. The same can be said about the content of Mn^{2+}, the manganese ions are evenly distributed in parts of the mass.

Tomograms of NMR and EPR showed that besides the factor of mechanical distribution of the powder in coarse disperse systems, the number of water components in this part of the system plays an important role. It is established that the number of spin tags correlates with the amount and time of water relaxation.

It is shown that for the uniform distribution of the powdered additive in coarse disperse systems, it is necessary to regulate powder dispersion and homogeneity of the system by the water content.

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ИЗУЧЕНИЕ ЗАКОНОМЕРНОСТЕЙ РАСПРЕДЕЛЕНИЯ ПОРОШКООБРАЗНЫХ ДИЕТИЧЕСКИХ ДОБАВОК В СОСТАВЕ ПИЩЕВЫХ ПРОДУКТОВ ГРУБОДИСПЕРСНОГО ТИПА

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Аннотация. В данной статье приведены примеры решения проблемы распределения динтических добавок в грубодисперсных системах. Серьезным вмешательством в состав продуктов питания считается обогащения пищевых продуктов микронутриентами. В связи с этим исследовано, как добавка с соответствующим микроэлементом будет распределяться в продукте питания, а в данном случае в мясном фарше, для того, чтобы удовлетворить потребности человека в микроэлементах.

Было проведено два анализа методом ядерного магнитного резонанса (ЯМР) и электронного парамагнитного резонанса (ЭПР) для определения распределения добавки в продуктах питания. Анализы проводились соответственно в два этапа: исследование подвижности молекул путем измерения времени спин-спиновой релаксации (T_2) и спин-решоточной релаксации (T_1) на импульсном спектрометре ЯМР; установление связи между показателем амплитуды образца A_0 и его массой. На основе полученных в результате измерения данных построено кривую зависимости амплитуды сигнала эха от величины (интервал времени между зондирующими импульсами). Спиновой меткой, которая использована в работе, является один из первых вариантов парамагнитного зонда – легкодоступный ион переходного металла Mn^{2+}. По построенными графиками и томограммами по результатам исследований изучали характеристику данной диетической добавки и целесообразность ее применения.

Ключевые слова: порошкообразные диетические добавки, ядерный магнитный резонанс, электронный парамагнитный резонанс.

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